B 606629
AUG 14 1956

VOLUME 14 NUMBER 8

NUTRITION REVIEWS

ONE COPY SENT TO L. C.

August 1956

AUG 23 1956

Amino Acid Imbalances, Toxicities and Antagonisms
By A. E. Harper 225

Clinical Nutrition
Hypoglycemia Associated with Tumors 228
The Obese High School Girl 229
Trace Elements in Enamel and Dentin 230
Amino Acid Requirements of Adult Man 232
Use of Sorbitol in Medicine 236

Animal Nutrition
Copper—A Growth Promotant? 238
Vitamin K and Sulfaquinoxaline Poisoning in Chickens 240
Effects of Antibiotics in the Ruminant 241

Experimental Nutrition
Nutrition Studies with Thermophilic Bacteria 243
Vitamin B_{12} and Sulfhydryl Enzymes 244
Vitamin A and Cholesterol Metabolism 245
Carbohydrate Metabolism of the Lens 246
Nutriture and Tobacco Smoke Lesions in Mice 248
Riboflavin Biosynthesis 250
The Distribution of Excessive Water 251
Function of Vitamin D 253

Notes
Dietary Fat and the Nephrotic Syndrome in Rats 255
Vitamin Retention in Frozen Foods 255
Recent Nutrition Books 256

Published Monthly at Mt. Royal and Guilford Aves., Baltimore 2, Md.

For

THE NUTRITION FOUNDATION, INC.

99 PARK AVE. NEW YORK 16, N. Y.

COPYRIGHT, 1956, NUTRITION FOUNDATION INC., NEW YORK, U. S. A.

Editor

FREDRICK J. STARE, PH.D., M.D., Professor of Nutrition, Schools of Medicine and Public Health, Harvard University; Associate in Medicine, Peter Bent Brigham Hospital, Boston. Editorial Office, One Shattuck Street, Boston, Mass.

Assistant Editor

CHRISTINE S. WILSON, One Shattuck Street, Boston

Associate Editors

GEORGE M. BRIGGS, PH.D., National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda
WILLIAM H. DAUGHADAY, M.D., School of Medicine, Washington University, St. Louis
JAMES S. DINNING, PH.D., Department of Biochemistry, University of Arkansas School of Medicine, Little Rock
R. W. ENGEL, PH.D., Department of Biochemistry and Nutrition, Virginia Polytechnic Institute, Blacksburg
ROBERT D. GAUCHAT, M.D., Department of Pediatrics, State University of Iowa, Iowa City
D. M. HEGSTED, PH.D., Department of Nutrition, Harvard School of Public Health, Boston
JAMES F. MEAD, PH.D., Department of Physiological Chemistry, School of Medicine, University of California, Los Angeles
OLOF H. PEARSON, M.D., Sloan-Kettering Institute, Memorial Center, Cornell University Medical College, New York
ROBERT E. SHANK, M.D., School of Medicine, Washington University, St. Louis
JAMES H. SHAW, PH.D., Schools of Dental Medicine and Public Health, Harvard University, Boston
ELMER H. STOTZ, PH.D., Department of Biochemistry, School of Medicine, University of Rochester, Rochester
ROBERT W. WISSLER, PH.D., M.D., Department of Pathology, University of Chicago

Editorial Committee

REGINALD M. ATWATER, M.D., American Public Health Assn.
WALLACE R. AYKROYD, M.D., Food and Agriculture Organization
GEORGE R. COWGILL, PH.D., Yale University
CHARLES S. DAVIDSON, M.D., Boston City Hospital
CONRAD A. ELVEHJEM, PH.D., University of Wisconsin
IAGO GALDSTON, M.D., New York Academy of Medicine
WENDELL H. GRIFFITH, PH.D., University of California
CHARLES GLEN KING, PH.D., Nutrition Foundation
L. A. MAYNARD, PH.D., Cornell University
HAZEL STIEBELING, PH.D., Bureau of Human Nutrition & Home Economics
LEROY VORIS, PH.D., National Research Council
WARREN WEAVER, PH.D., Rockefeller Foundation
JOHN B. YOUMANS, M.D., Vanderbilt University

Subscriptions should be sent to The Nutrition Foundation, Inc., 99 Park Ave., New York 16, N. Y. Rate $4.00 per year in the United States and Canada; $4.50 in other countries.
Changes of address should be sent to The Nutrition Foundation.
Entered as second-class matter at the Post Office at Baltimore, Md.

Note: Items may be quoted from NUTRITION REVIEWS without special permission. If quotations are made, the editors ask only that credit be given to NUTRITION REVIEWS and that they be made in such a way that the original meaning of the review is preserved.

B 606629

AUG 14 1956

NUTRITION REVIEWS

VOL. 14 AUGUST 1956 No. 8

AMINO ACID IMBALANCES, TOXICITIES AND ANTAGONISMS

It is just fifty years since Willcock and Hopkins discovered that a supplement of tryptophan prolonged the survival of rats fed on diets containing the nutritionally inadequate corn protein, zein. Since then our knowledge of the nutritional role of amino acids has been extended until now supplements of crystalline amino acids are commonly used for the improvement of experimental diets and serious consideration is being given to their use on a practical scale. It is perhaps appropriate, then, to recall that several of the indispensable amino acids can be quite toxic when fed at levels of from two to five times the requirement, and that undesirable effects have been observed from time to time when relatively small amounts of individual amino acids have been added to inadequate diets.

Any attempt to classify the various detrimental effects that have been encountered is beset with difficulties and, until a satisfactory explanation is found in each case, only the loosest sort of grouping is justifiable. Among the more general terms that have been used to describe these effects are *imbalance*, *toxicity* and *antagonism*. Of these, the term imbalance has been the most widely, and perhaps the most loosely, used. This is understandable because any alteration in the amino acid composition of a diet, except a proportional change in the levels of all the amino acids, is accompanied by a change in the amino acid balance. Thus almost any detrimental effect from adding an amino acid to a diet can be attributed to the creation of an amino acid imbalance. The question arises, however, whether this term should be used in such a general way.

The concept of an amino acid imbalance arose from experiments on niacin deficiency when a supplement of a tryptophan-deficient protein, such as gelatin, was found to depress the rate of growth of rats fed a diet that lacked niacin and was low in tryptophan (W. A. Krehl, L. J. Teply, and C. A. Elvehjem, *Science* **101,** *283* (*1945*)). The imbalance, which could be prevented by a supplement of either niacin or tryptophan, was subsequently traced to an increase in the level of threonine (Krehl, L. M. Henderson, J. de la Huerga, and Elvehjem, *J. Biol. Chem.* **166,** *531* (*1946*)), valine, or lysine (Henderson, O. J. Koeppe, and H. H. Zimmerman, *Ibid.* **201,** *697* (*1953*)), when each of these was the second most limiting amino acid for growth. Since the imbalance has been observed when adequate niacin is provided, the reversal by niacin apparently depends on its ability to spare tryptophan (W. D. Salmon, *Arch. Biochem. Biophys.* **51,** *30* (*1954*)).

There are a number of other cases in which a supplement of the second most limiting amino acid for growth causes a more severe deficiency of the most limiting. Among these may be included an imbalance induced by lysine in a diet deficient primarily in histidine (M. E. Winje and co-workers, *J. Nutrition* **54,** *155* (*1954*)), and an imbalance induced by valine in a diet deficient primarily in threonine (B. Sure, *J. Agr. Food Chem.* **2,** *1108* (*1954*)). A series of imbalances has been observed when rice diets have been supplemented in various ways (L. J. Pecora and J. M. Hundley, *J. Nutrition* **44,** *101* (*1951*); A. E. Harper, Winje, D. A. Benton, and C. A. Elvehjem, *Ibid.* **56,** *187* (*1955*)) and recently C. F. Gessert and P. H. Phillips (*Ibid.* **58,** *423* (*1956*)) at Wisconsin, using the dog as a test animal, have demonstrated a similar imbalance involving methionine and lysine. A decreased rate of growth is not the only criterion of an amino acid imbalance. An increase in the deposition of liver fat, which is prevented by a supplement of threonine, has been observed when a small amount of methionine

is added to a diet that is low in threonine (Harper, Benton, Winje, and Elvehjem, *J. Biol. Chem.* **209,** *159 (1954)*).

Imbalances which retard growth can be most simply explained if, as Salmon has suggested for incomplete proteins, a supplement of the second most limiting amino acid leads to an increased rate of catabolism of all amino acids, and thereby to an increased loss of the most limiting. This can scarcely be the whole answer, however, because, if it were, any amino acid supplement should produce a similar effect. The example involving liver fat deposition suggests that the proportions of amino acids required for specific physiologic processes may differ from those required for growth, and that a stimulation of growth may make less of a limiting amino acid available for some specific process, such as the control of liver fat deposition. Regardless of the validity of these explanations, the resemblance of the examples mentioned to the original niacin-tryptophan imbalance would seem to be sufficient reason for restricting the use of the term "amino acid imbalance" to cases which satisfy the following criteria: (1) a detrimental effect (*e.g.*, growth depression) is observed when the diet of animals receiving a suboptimal supply of essential amino acids is supplemented with amino acids or protein; (2) this effect is prevented by a small supplement of the amino acid that is most limiting for the process being studied.

This definition would exclude those cases in which a dietary excess of an amino acid increases the severity of deficiencies of such vitamins as pyridoxine or vitamin B_{12}. These cases would seem to be analogous to that described by W. H. Riesen, B. S. Schweigert, and C. A. Elvehjem (*Arch. Biochem.* **10,** *387 (1946)*) in which an increase in the level of dietary protein increased the severity of a riboflavin deficiency, presumably because the need for a vitamin involved in amino acid metabolism is increased when more amino acids are ingested.

Another example that would fall outside the range of this definition is that in which a vitamin deficiency, previously masked because growth was limited by a lack of protein, is made evident or more severe by increasing the nutritive value of the dietary protein. The case of a choline deficiency is complicated because methionine serves as a source of methyl groups for choline synthesis. However, unlike the amino acid imbalance, no detrimental effect from the amino acid supplement can be demonstrated if adequate choline is provided.

The retardation of growth and other detrimental effects that result from feeding excessive amounts (two to ten times the requirement) of single amino acids have usually been attributed to amino acid toxicities. Such an effect was observed at least thirty years ago (H. B. Lewis, *J. Biol. Chem.* **65,** *187 (1925)*), and since then excessive amounts of most of the indispensable and many of the dispensable amino acids have been shown to cause toxic reactions, particularly in animals fed diets that are low in protein or deficient in a vitamin. Recently, leucine in excess was found to act as an antagonist of isoleucine (A. E. Harper, D. A, Benton, and C. A. Elvehjem, *Arch. Biochem. Biophys.* **57,** *1 (1955)*; *Nutrition Reviews* **14,** *20 (1956)*), and a more complex antagonism involving leucine, isoleucine and valine has since been demonstrated (Benton, Harper, H. E. Spivey, and Elvehjem, *Arch. Biochem. Biophys.* **60,** *147 (1956)*). It is unlikely, however, that toxic effects of excessive amounts of amino acids will prove generally to be the result of amino acid antagonisms. This becomes evident if methionine toxicity, which has been studied in some detail, is taken as an example. The toxicity is alleviated when arginine and glycine or glycocyamine are added to the diet (D. S. McKittrick, *Arch. Biochem.* **15,** *133 (1947)*; J. S. Roth, J. B. Allison, and L. J. Milch, *J. Biol. Chem.* **186,** *113 (1950)*). Conversely, large amounts of arginine and glycine or glycocyamine cause increased accumulation of liver fat in

rats receiving diets in which methionine is substituted for choline (Harper and Benton, *Biochem. J.* **62,** *440 (1956)*). These observations suggest that a shift in the equilibrium of some metabolic reaction is the basis for the detrimental effects of excessive amounts of these amino acids. Thus mechanisms such as competitive inhibition of specific enzymic reactions through antimetabolite action, accumulation of harmful products of metabolism which cannot be eliminated at a sufficiently rapid rate, and the depletion of some essential component required for the metabolism of the amino acid provided in excess may be three of a variety of causes of amino acid toxicities.

Although excessive amounts of amino acids can produce toxic effects in animals receiving adequate protein, imbalances and antagonisms are most readily demonstrated in animals fed on diets low in protein. Antagonisms can be differentiated from imbalances, because an amino acid which acts as an antagonist must be provided in amounts that considerably exceed the requirement and the amino acid against which it is an antagonist does not have to be limiting for growth. The demonstration of these effects probably depends more on the relative proportions of the dietary amino acids than on the actual amount of dietary protein. It is interesting that the amino acid requirements of chicks increase as the level of dietary protein is increased (H. J. Almquist, *Poultry Sci.* **31,** *966 (1952)*). Since the only way such information can be obtained is by having one amino acid in the diet limiting for growth, this effect may be the result of an amino acid imbalance at a higher protein level. It is, of course, also possible that antagonisms or other toxic effects may be induced under such circumstances by high levels of protein.

Interpretation of the effects of amino acid supplementation can be complicated by a lack of quantitative information about the availability of amino acids in proteins, particularly the alcohol-soluble proteins of cereals. Zein, for example, contains a sufficient excess of leucine to cause an isoleucine antagonism and rats fed on diets containing corn or zein as the source of protein show a striking response to isoleucine supplements. Until the availability of these two amino acids can be accurately estimated, however, it is not possible to determine whether an antagonism is of significance (D. A. Benton, A. E. Harper, and C. A. Elvehjem, *Arch. Biochem. Biophys.* **57,** *13 (1955)*; *Nutrition Reviews, loc. cit.*). Also, if the amino acids of rice are listed from analytic data in the order in which they become limiting for growth, only the least limiting and the most limiting occupy the same position as they do when measured by biologic assay, and imbalances can be unwittingly created because of the lack of this information (P. D. Deshpande, Harper, F. Quiros-Perez, and Elvehjem, *J. Nutrition* **57,** *415 (1955)*).

The concept that amino acids should be provided in a diet in proportions paralleling the requirements of the animal is undoubtedly basically sound, but a multitude of questions remains to be answered. To what extent can this balance be altered without leading to injurious effects? To what extent does the balance of amino acids required for other bodily functions differ from that required for growth generally, particularly when animals are fed on diets that do not support optimum growth rates? To what extent are the amino acids in diets composed largely of crude food materials available to the animal? Although these are complex problems which will occupy nutritionists for many years to come, the need for answers to them becomes more urgent as methods are developed which will permit large-scale production of amino acids for practical supplementation programs.

A. E. HARPER
Department of Biochemistry
University of Wisconsin
Madison

HYPOGLYCEMIA ASSOCIATED WITH TUMORS

The association of severe hypoglycemia with large abdominal tumors has been noted sporadically throughout the literature. A recent case reported by R. S. Silvis and D. S. Simon (*New Engl. J. Med.* **254,** *14 (1956)*) serves to focus attention again on this syndrome and to raise a number of speculations. The patient was a 23 year old male who, during episodes of negativism and mental confusion, was found to have a blood sugar of 35 mg. per cent. The symptoms responded quickly to intravenously administered glucose. Subsequently, a 1200-gram retroperitoneal tumor was removed. Following surgery there was no recurrence of the attacks.

Similar cases were reported by H. A. Arkless (*Med. Bull. Vet. Admin.* **19,** *225 (1942)*) and R. E. Hines (*Ibid.* **20,** *102 (1943)*). In both instances, the patients were men. The tumors were large (2700 and 3500 g.), and both arose retroperitoneally. In each instance, the symptoms of hypoglycemia were controlled by intravenous administrations of sugar. The first patient died suddenly before surgery; the second had recurrence of hypoglycemia after resection of the tumor and died from metastatic spread of the tumor.

P. G. Skillern, L. J. McCormack, J. S. Hewlett, and G. Crile (*Diabetes* **3,** *133 (1954)*) discussed 2 similar instances. The patients were a 68 year old woman and a 70 year old man. The tumor weights were in the range previously noted. Surgical removal of these tumors led to relief of the syndrome.

In the 5 cases discussed above, the histologic appearance of the tumors suggested that they were sarcomas with fibrosis. The cells were usually described as being spindle-shaped, some of them being pleomorphic (having different forms) and giving the appearance of malignancy. As none of these tumors has been assayed for insulin, one can but speculate regarding the effect of these tumors on the blood sugar level. It has been suggested that, in view of the size of these tumors, excessive utilization of sugar by the tumor tissue might be suspected. This is hardly a tenable argument in the face of numerous examples of patients with larger amounts of tumor tissue and no hypoglycemia.

Skillern *et al.* (*loc. cit.*) have made two suggestions. They consider the possibility that these cells represent transformation of the epithelial-like round cells of the islets of the pancreas. If they arise from islet cells, then presumably it would be from ectopic pancreatic tissue. However, only 6 cases of islet cell adenomas arising from ectopic pancreatic tissue have been recorded. There remains the possibility that these tumors arise from a more primitive retroperitoneal tissue which differentiates sufficiently to produce insulin. This latter explanation seems more probable for the patients just discussed, but hypoglycemia has also been noted in patients with other tumors (*see below*), and may depend upon other mechanisms.

H. B. Anderson (*Am. J. Med. Sci.* **180,** *71 (1930)*) noted the classic syndrome of hyperinsulinism in a 33 year old male who was subsequently found to have a carcinoma of the left adrenal gland. The cells of the tumor resembled those of adrenal cortical tissue. C. H. Lawrence (*Ann. Int. Med.* **11,** *936 (1937)*) discovered hypoglycemia in a 24 year old woman with masculinization and hypertension. She, too, was found to have a carcinoma of the adrenal cortex. J. J. Staffieri, O. Cames, and J. M. Cid (*J. Clin. Endocrinol.* **9,** *255 (1949)*) reported the association of hypoglycemia with a malignant tumor arising in the region of the right adrenal gland and composed of sarcomatous cells. L. R. Broster and J. Patterson (*Brit. Med. J.* **1,** *781 (1948)*) discovered a left adrenal tumor in a 14 year old girl

with virilism and attacks of hypoglycemia. In none of these 4 patients was there ancillary evidence of adrenal hypofunction or any suggestion of gross destruction of the adrenal gland. Indeed, in the last instance cited, the urinary 17-ketosteroids were 1980 mg. per day, so that this was a functioning adrenal cortical tumor. E. D. Rosenfeld (*Arch. Path.* **48,** *255 (1949)*) described 2 instances of peritoneal pseudomyxoma with hypoglycemia. In one of these, the response to oral glucose and to a fast was typical of hyperinsulinism.

The diversity of pathologic material in this last group of patients argues against the hypothesis that the tumors produce insulin or an insulin-like material. Unfortunately, at the time of autopsy, there was in no case a thorough search of the pancreas for functioning adenomas. The problems connected with this rare association of hypoglycemia and nonpancreatic tumors will remain largely speculative until insulin assays are performed on these tumors, and the possible coincidence of pancreatic adenomas is ruled out.

THE OBESE HIGH SCHOOL GIRL

Obesity in the adult is an unfortunate phenomenon and a well-recognized potential danger to the health of the individual concerned. Obesity in a girl of high school age, however, is more than a threat to future well-being—it is an immediate personal problem that the adolescent girl feels keenly when, as so often happens, she observes how much more successful her slimmer classmates are in social popularity. Recent studies by M. L. Johnson, B. S. Burke, and J. Mayer (*Am. J. Clin. Nutrition* **4,** *37 (1956)*) on the relative importance of inactivity and overeating in the energy balance of obese high school girls provide information on some of the important physiologic differences that exist between fat girls and their slimmer contemporaries.

Two groups of high school girls—28 obese and 28 nonobese—of similar height, age and school grade were compared with particular regard to their physical maturation, food intake and activity. The obese girls showed an earlier deceleration in height and a tendency toward earlier menarche, and this was regarded as indicative of accelerated development and earlier maturation. As regards activity, "perhaps the most striking impression is that these suburban high school girls are not physically active. Even the schedules of the nonobese group show little time devoted to household chores, little participation in active sports, and, at least during the school term, a minimum amount of time devoted to walking or other physical activity. Available facilities for sports are little used. However, even when the generally low level of activity is considered, the method of analysis used in this study still allows for the semiquantitative demonstration of clear-cut differences between the nonobese and obese groups." The activities of these girls were represented by a system of "activity indices." Ratings of energy expenditure for different activities, based on estimations of caloric expenditure above basal, were obtained from the tables of Rose (C. M. Taylor and G. MacLeod, "Rose's Laboratory Handbook for Dietetics," *5th edition, Macmillan, New York (1949)*) and of J. B. Orr and I. Leitch (*Nutrition Abstr. Rev.* **7,** *509 (1938)*), and were multiplied by the total hours spent each week in each activity. The products totaled and divided by 7 gave the "activity index" per day.

Comparison of caloric intakes and activity indices showed that "although the obese group was not homogeneous with regard to the type of energy balance by which they achieved obesity," inactivity was more important in the development and main-

tenance of obesity than "overeating"; indeed, the obese group had a significantly lower intake of calories than did the non-obese. The fact remained, of course, that they were overeating in relation to their low energy expenditure.

Although there was no significant difference between the average weights of the fathers of the obese and control girls, 42 per cent of the mothers of the obese girls were above "ideal" weight as defined by the Metropolitan Life Insurance Company (*Metropolitan Life Insurance Co. Statist. Bull.* **23,** *No. 10, 6 (1942)*), compared with only 7 per cent of the mothers of control girls; this difference was highly significant. This finding recalls the "obesity cycle" described by E. M. Widdowson (*Am. J. Clin. Nutrition* **3,** *391 (1955)*). Miss Widdowson observed that "there is no doubt that the fat mother tends to have a large fat baby"; further, "a big baby born of a fat mother seems to be endowed with something which makes it continue to eat in proportion to its size. This may be growth hormone, but whatever it is, it keeps these children ahead of their fellows. They eat a little more; they may or may not get fat during the growth period, but whether they do or not, they tend to reach puberty at an early age and a heavy weight."

One minor criticism of these studies of Johnson *et al.* is the subjective nature of the information on which assessment of the energy demands of the different activities indulged in by the girls was made. Now that such a convenient instrument as the Kofrányi-Michaelis respirometer (*see Nutrition Reviews* **12,** *196 (1954)*) is available and has been used for determining the energy demands of a variety of occupations, many of them far more energetic (*see Ibid.* **14,** *48 (1956)*) than that of the inactive high school girls studied by Johnson *et al.*, it seems a pity not to make use of such a device in studies of the present kind.

TRACE ELEMENTS IN ENAMEL AND DENTIN

The elemental composition of enamel and dentin has become a fertile field for investigation during recent years. Since minor components, such as fluorine, have been shown to vary in proportion to the amount available during the calcification process and since a strong correlation exists between the fluoride content of these tissues and their susceptibility to tooth decay, the search has been extended to other phases of the fluoride problem and also to other elements (*Nutrition Reviews* **10,** *76 (1952)*).

In an effort to determine the mechanism of action of fluorides in enamel, G. N. Jenkins and R. L. Speirs (*J. Physiol.* **121,** *21 P (1953)*) studied the fluoride content at different distances from the surface of the enamel of teeth from individuals living in areas of England with different fluoride levels in the water supplies. Surface enamel from teeth of subjects in West Hartlepool where the water contained 2.0 parts per million (p.p.m.) of fluoride had between 113 and 180 p.p.m. of fluoride whereas the deeper enamel contained only 27 p.p.m. In South Shields, with a water supply containing 1.4 p.p.m. of fluoride, the content in the surface and inner enamel was 96 and 11 p.p.m., respectively. In North Shields, where the water has less than 0.25 p.p.m. of fluoride, the content in the surface and inner enamel was 61 and 8 p.p.m. of fluoride.

The above values were all obtained with erupted teeth. In order to test whether the higher levels of fluoride in the surface enamel had been attained after eruption into the oral cavity by adsorption of fluoride from drinking water and oral fluids, these authors studied the distribution of fluorides in unerupted teeth. They came to the conclusion that a large part of the difference in fluoride content between the surface and inner layers of enamel was determined during the calcification period.

Few samples were involved in the above

study. However, the observations of Jenkins and Speirs are strengthened by data presented by F. Brudevold, D. E. Gardner, and F. A. Smith (*J. Dent. Res.* **33,** *650 (1954)*), who reported that external enamel of erupted human teeth had a fluoride concentration that was six to ten times greater than that of the remaining bulk of enamel. The surface level of fluoride of the teeth in a group of subjects under 20 years of age was 600 p.p.m. and went as high as 800 to 1000 p.p.m. in older age groups. A high concentration of fluoride was also found in the external enamel of impacted teeth, which suggests that appreciable amounts of fluoride were picked up by the superficial enamel prior to eruption. The relative distribution of fluoride in the different layers of fluorosed enamel was comparable to that in nonfluorosed teeth, but the concentration of fluoride in each of the different layers was about twice as great.

Numerous investigators have reported the presence of copper in dental tissues with rather wide ranges. In one investigation, M. M. Ginzburg and I. E. El'piner (*Stomatologia* **1,** *13 (1947)*) suggested that there was a correlation between the amount of copper and the integrity of the teeth because they observed 5.2 micrograms of copper per gram of ash in sound teeth and 18.5 micrograms of copper per gram of ash in carious teeth. In a more intensive study, F. Brudevold and L. T. Steadman (*J. Dent. Res.* **34,** *209 (1955)*) have examined the relationship of copper to various dental problems. The copper content in 4 successive layers of human enamel was obtained by grinding off these layers with a diamond stone. Samples were taken from age groups under 20, from 20 to 29 years of age, 30 to 49, and over 50. Teeth that were mottled and others that were hypoplastic were included in the study. The copper determinations were made by means of spectrum analyses with a Bausch and Lomb medium quartz spectrograph.

The average copper content of enamel was found to be 20.5 p.p.m., with a range from 12 to 30 p.p.m. No definite pattern of distribution of copper was observed in different areas in the enamel. Likewise, the copper content of the teeth did not change detectably with increasing age of the individual and there was no detectable difference between the enamel of unerupted impacted teeth and that of erupted teeth. The latter two facts would indicate that the copper content did not change appreciably after eruption regardless of the length of time in the oral cavity. It was interesting to note that the copper content of fluorosed enamel was somewhat lower than for enamel that was not fluorosed. A wide variation was observed in the copper content of surface enamel from different teeth. No correlation was found between the copper content and enamel solubility, pigmentation, hypoplasia, or dental caries.

F. Brudevold and L. T. Steadman (*J. Dent. Res.* **34,** *674 (1955)*) have studied the content of lead and tin in different layers of human enamel in both unerupted and erupted teeth of different ages. Lead was found to be present in all groups of teeth, occurring in greatest concentration in the outer enamel and decreasing in concentration in the inner layers, to a level in the neighborhood of 30 to 90 p.p.m. The lead concentration in the outer layers of fully formed unerupted teeth was greater than that in the outer layers of incompletely formed teeth; a further increase appeared in the outer enamel of erupted teeth. Tin was found in concentrations of 1 p.p.m. and was evenly distributed through the enamel of unerupted teeth. It is interesting to note that a greater tin concentration was found in teeth filled with amalgam than in enamel from intact teeth.

M. F. Little and F. Brudevold (*J. Dent. Res.* **34,** *708 (1955)*) have studied the distribution of inorganic carbonate in human enamel. They found that the concentration was lowest in the outer layer of the enamel and increased to levels as high as 2.3 to 2.4 per cent in the subsurface enamel. The car-

bonate content of superficial enamel was routinely higher in unerupted teeth than in erupted teeth.

In another investigation G. W. Burnett and R. R. Lobene (*J. Dent. Res.* **34,** *814 (1955)*) have made spectrochemical analyses of the enamel and dentin of Syrian hamsters for 34 elements. The following 17 elements were found consistently in incisor and molar enamel and are arranged here in descending order of their magnitude of occurrence in molar enamel: calcium, phosphorus, sodium, magnesium, strontium, silicon, barium, potassium, manganese, chromium, copper, iron, aluminum, boron, lithium, silver and lead. In addition, tin was found in the incisor and molar enamel of males but not of females, and in the incisor dentin of males and females but not in molar enamel or dentin of either sex. Nickel was observed in molar enamel of males and females, but not in the incisor enamel of either sex and not in the dentin of either incisors or molars of either sex. Gold, beryllium, bismuth, cadmium, cobalt, cesium, gallium, germanium, indium, molybdenum, rubidium, antimony, titanium, zinc and zirconium were not observed in any of the tissues within the range of the estimated limits of the procedure.

At the present time the above studies provide us with a series of isolated facts from which it is impossible to draw any hypothesis or realistic concept of the purpose of all these elements in enamel and dentin or the reason why some elements such as lead and fluoride should have higher levels in the surface enamel than in the inner enamel, whereas the carbonate content exhibits the reverse trend. Whether any of these elements except fluoride is present for any other reason than its adventitious occurrence in the blood stream during calcification is not known.

One cannot help pondering the merits of intensive investigation of these subjects. With the availability of satisfactory methods for the evaluation of the composition of the calcified tissues with respect to these numerous elements and also the increasing ability to produce diets that are very low in these elements, wide areas for study have become accessible. There are numerous interesting aspects to these studies not only regarding the composition and maintenance of the teeth themselves, but also the growth, development and calcification of the bones and teeth, and the susceptibility of bones to such phenomena as osteoporosis and increased fragility in older age groups.

AMINO ACID REQUIREMENTS OF ADULT MAN

Results of the classic studies of Rose and co-workers on the amino acid requirements of adult man are well known (W. C. Rose, *Fed. Proc.* **8,** *546 (1949)*; *Nutrition Reviews* **8,** *118 (1950)*). Complete details of the experiments, with additional information, have now been published.

As has been previously reported, only eight amino acids, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine are required to maintain nitrogen equilibrium in the diet of adult man. Only the L-isomers are active, with the exception of D-methionine, which is fully as active as the L-isomer, and D-phenylalanine, which is active but to a lesser degree than the L-isomer.

Rose and co-workers (W. C. Rose, G. F. Lambert, and M. J. Coon, *J. Biol. Chem.* **211,** *815 (1954)*; Rose, R. L. Wixom, H. B. Lockhart, and Lambert, *Ibid.* **217,** *987 (1955)*), in establishing the quantitative minimal requirements, have been careful to call such requirements only tentative because of the relatively small number of subjects used (a total of 38 short-term quantitative experiments extending over a period of twelve years). Experimental subjects were healthy male graduate students given a diet consisting of wafers of cornstarch, sucrose,

butterfat, corn oil and inorganic salts, supplemented with cellu flour, water and lemon-flavored aqueous mixtures of recrystallized amino acids. Vitamins and a butyl alcohol extract of liver powder were given daily. No biotin, choline, vitamin B_{12}, or zinc was given except what may have occurred as impurities in the dietary ingredients (such as filtered lemon juice or liver extract). To the amino acid supplements were added glycine, urea, or both, as further sources of nitrogen.

The total nitrogen intake on such diets was 6.7 to 10.1 g. per day, including the D-isomers of some of the amino acids. One third of the daily allotment of amino acids and other dietary constituents, excepting the vitamins, was consumed at each meal. The vitamins were taken either once daily or at the morning and evening meals. Some difficulty was created by the monotony of the diets and the unattractive taste of the amino acid solutions. These circumstances caused a few subjects to abandon tests before completion.

Sufficiency of the dietary regimen was established by determinations of nitrogen balance. As has been pointed out (*Nutrition Reviews* **13,** *71 (1955)*), about 25 per cent more calories were required to maintain positive nitrogen balance with amino acid mixtures than with intact protein. For this reason the caloric content of all diets was routinely maintained at 55 calories per kilogram of body weight per day. Somewhat lower intakes might have sufficed in some instances, but the higher values "diminished the length of the adjustment periods and induced nitrogen equilibrium much more promptly than would have occurred otherwise," according to the authors.

The minimal amino acid requirement was determined by a stepwise decrease in the amino acid level over successive periods of three to ten days each until a negative balance, as measured by the average for the period, was obtained. The diet was then supplemented with the amino acid until a distinctly positive balance was attained. The minimal daily intake of the amino acid that would maintain positive nitrogen balance was determined for each subject. By the authors' definition, the highest observed value for all the subjects (rather than an average) was designated as the "tentative minimal requirement." This gave allowance for biologic variability and for the small number of individuals.

The *isoleucine* and *leucine* requirements were determined by W. C. Rose, C. H. Eades, and M. J. Coon (*J. Biol. Chem.* **216,** *225 (1955)*). In the 4 subjects tested the minimal daily requirement for L-isoleucine ranged from only 0.65 to 0.7 g. per day, with 0.7 g. set as the tentative minimal daily requirement. The exclusion of isoleucine from the diet was followed immediately by a negative balance of greater magnitude than that encountered in any other amino acid deficiency in man, according to the authors. The results with leucine deficiency were quite in contrast to the consistent isoleucine results. In 5 subjects the minimal daily requirement for L-leucine ranged from 0.5 to 1.1 g., which was the most pronounced variation encountered in any of the amino acids. The minimal daily requirement was set tentatively at 1.1 g. per day. No correlation could be detected between the minimal needs of each subject and his body weight, surface area, or creatinine output, which was true for all the amino acids.

The *lysine* requirement was studied in 6 subjects and found to be 0.8 g. per day, with a range of from 0.4 to 0.8 g. (W. C. Rose, A. Borman, M. J. Coon, and G. F. Lambert, *J. Biol. Chem.* **214,** *579 (1955)*). No changes in the urinary output of organic acids were seen in these experiments, in contrast to results obtained in lysine-deficient human subjects by A. A. Albanese *et al.* (*Proc. Soc. Exp. Biol. Med.* **52,** *209 (1943)*). Also, as in all the amino acid deficiencies, no specific subjective symptoms were encountered; however, all the subjects experienced a loss of appetite, an increase in nervous irritability, and a sensation of fatigue prevented by feeding the missing amino acid.

The tentative minimal *methionine* re-

quirement, as determined in 6 subjects, was set at 1.1 g. per day (W. C. Rose, M. J. Coon, H. B. Lockhart, and G. F. Lambert, *J. Biol. Chem.* **215,** *101* (*1955*)). The D-form of methionine was fully as active as the L-form, even when given alone. A surprising finding was the extent to which L-cystine was able to replace the methionine requirement in 3 subjects tested (Rose and R. L. Wixom, *Ibid.* **216,** *763* (*1955*)). These workers reported that L-cystine is capable of replacing 80 to 89 per cent of the minimal methionine needs of adult man. This is considerably greater than the cystine-sparing effect in the weanling rat (about 33 per cent) and the chick (30 to 50 per cent). In other words, in the presence of ample cystine only 0.1 to 0.2 g. of methionine was needed by the adult male to maintain nitrogen balance over a period of at least five days. The authors point out that in "those areas of the world in which native diets are low in methionine, little consideration appears to have been given to the beneficial effect of cystine." They suggest that such diets should be re-evaluated in regard to their total methionine and cystine content. The possible sparing effect of choline and of vitamin B_{12} on the methionine requirement was not studied.

The tentative minimal *phenylalanine* requirement was found to be 1.1 g. per day, as determined in 6 individuals (W. C. Rose, B. E. Leach, M. J. Coon, and G. F. Lambert, *J. Biol. Chem.* **213,** *913* (*1955*)). Although a positive nitrogen balance was obtained with 1.1 g. of DL-phenylalanine, the D-form alone, at 1.1 or 1.5 g. per day, did not produce a positive balance. This indicated that about 0.50 to 0.55 g. per day of D-phenylalanine, but no more than this amount, could replace the L-form. In 2 subjects it was found that L-tyrosine is capable of exerting a sparing effect of from 70 to 75 per cent upon the phenylalanine needs of adult man (Rose and R. L. Wixom, *Ibid.* **217,** *95* (*1955*)). This again is a surprising finding, the extent being somewhat larger than the ability of tyrosine to spare phenylalanine in the weanling rat. In the presence of ample tyrosine the 2 subjects maintained positive nitrogen balance on a daily intake of only 0.3 g. of phenylalanine.

The *threonine* requirement was found to range in value from 0.3 to 0.5 g. per day, with a tentative daily minimal value of 0.5 g. in studies with 3 subjects (Rose, Coon, Lockhart, and Lambert, *loc. cit.*).

The minimum level of *tryptophan* required in 3 subjects was 0.15, 0.15, and 0.25 g. per day, respectively (Rose, Lambert, and Coon, *loc. cit.*). Although 15 additional subjects were maintained in positive nitrogen balance with only 0.2 g. of tryptophan per day, the tentative daily minimal level was selected, by definition, as 0.25 g. per day.

The eighth essential amino acid for adult man, *valine*, was studied with 5 subjects (Rose, Wixom, Lockhart, and Lambert, *loc. cit.*). The minimum requirement was set at 0.8 g. per day, with values ranging from 0.4 to 0.8 g. This wide range was similar to those obtained with lysine and leucine.

In agreement with their earlier work, Rose and co-workers could find no requirement of adult males for arginine, histidine, or other amino acids by the nitrogen balance technic (W. C. Rose, W. J. Haines, and D. T. Warner, *J. Biol. Chem.* **206,** *421* (*1954*); Rose, Wixom, Lockhart, and Lambert, *loc. cit.*) A large number of subjects has been maintained in positive nitrogen balance for over a month on amino acid mixtures devoid of arginine and histidine without signs of deficiency. Whether these two amino acids would be more effective, quantitatively, than glycine and urea in supplying nitrogen needs for nonessential amino acid synthesis was not tested.

In previous studies (*see Nutrition Reviews* **8,** *118* (*1950*)) Rose had deemed it advisable to set the "recommended daily intake" at twice the value found for the minimal daily requirement because of the small number of subjects tested and because of the relatively

wide variation found between individuals in certain of their amino acid requirements. This provided an adequate margin of safety to cover any normal situation. This terminology has now been changed by Rose and his group because it had been interpreted by some as being synonymous with "optimal intakes." Rose and his co-workers now refer to twice minimal values as "safe intakes" and state ". . . one can show statistically that the odds are overwhelmingly against the possibility of a deficiency in a subject who is consuming twice the highest observed minimal requirement" (Rose, Lambert, and Coon, *loc. cit.*). Further evidence for this is offered in each of their studies in which from 17 to 42 subjects were maintained in positive nitrogen balance on "safe intakes" or less for each essential amino acid. The authors also state that a " 'safe intake' is not to be confused with an 'optimal intake,' which undoubtedly is still higher, but for which no direct measurement is available."

In summary, the tentative minimal daily requirements, the ranges observed, and the definite safe daily intakes of essential amino acids in adult man as determined by Rose and co-workers are as follows:

One valid criticism of their program which is fully acknowledged by the authors is the comparatively small sample of the human race which can be subjected to quantitative study, especially in tedious experiments of this nature. It is also recognized that many facets of the problem of amino acid requirements of adult human beings remain to be studied, including the effect of wide variations in body size, the effect of various stress conditions (such as disease, colds, exercise, old age, and hot or cold weather), and the effect of other dietary components (calories, vitamins, minerals, etc.). In spite of the obvious difficulties involved in making such tests, one wonders whether adult women, especially during pregnancy, have similar requirements and if studies extended over a longer period of time, based on nitrogen balance technics, might show different requirements, both qualitatively and quantitatively (*see Nutrition Reviews* **13,** *42 (1955)*). However, the important studies of these authors now give a strong base line from which future protein and amino acid experiments with man can be made.

Amino Acid	No. of Quantitative Experiments	Range of Requirements Observed	Proposed Tentative Minimum	"Safe Intake"
		g. per day	*g. per day*	*g. per day*
L-Isoleucine	4	(0.65–0.70)	0.70	1.40
L-Leucine	5	(0.50–1.10)	1.10	2.20
L-Lysine	6	(0.40–0.80)	0.80	1.60
DL-Methionine[1]	6	(0.80–1.10)	1.10[1]	2.20[1]
L-Phenylalanine[2, 3]	6	(0.80–1.10)	1.10[2, 3]	2.20[2, 3]
L-Threonine	3	(0.30–0.50)	0.50	1.00
L-Tryptophan	3	(0.15–0.25)	0.25	0.50
L-Valine	5	(0.40–0.80)	0.80	1.60
Totals	38		6.35 g.	12.70 g.

[1] L-cystine may replace up to 80 to 89 per cent of the minimal methionine needs. D-methionine is fully as active as L-methionine.

[2] L-tyrosine may replace up to 70 to 75 per cent of the minimal phenylalanine requirement.

[3] About 0.50 to 0.55 g. of D-phenylalanine can be utilized per day. Thus D-phenylalanine alone will not fulfil phenylalanine requirements. However, DL-phenylalanine will satisfy the minimum daily requirement at a level of 1.1 to 1.2 g. Satisfaction of the safe intake would require 2.75 g. of DL-phenylalanine per day (2.20 plus 0.55 g.).

USE OF SORBITOL IN MEDICINE

Sorbitol is a sugar alcohol which occurs naturally in certain edible fruits, vegetables and berries. It is a substance of considerable physiologic interest since it resembles glucose very closely in its molecular structure and yet is not capable of being utilized by extrahepatic cells. A. N. Wick and D. R. Drury (*Am. J. Physiol.* **166,** *421 (1951)*) have shown that C^{14}-labeled sorbitol, when administered intravenously to nephrectomized, eviscerated rabbits, has a volume of distribution in the extrahepatic tissues corresponding to the extracellular compartment. Unlike glucose and galactose, sorbitol does not enter the cell, with or without insulin action. Furthermore, sorbitol is not metabolized by hepatectomized animals, as demonstrated by isotopic labeling.

It is of interest, however, that ingested sorbitol is metabolized by the alloxan-diabetic rat almost as readily as by the normal rat (A. N. Wick, T. N. Morita, and H. N. Barnet, *Food Research* **20,** *66 (1955)*). R. L. Blakley (*Biochem. J.* **49,** *257 (1951)*) has demonstrated the presence in rat liver of an enzyme (sorbitol dehydrogenase) which converts sorbitol to fructose. Accordingly, the belief has been strengthened that sorbitol absorbed from the digestive tract is converted to glycogen and glucose by way of fructose.

When sorbitol is administered intravenously to dogs, only moderate levels of sorbitol in the blood are observed but a prompt elevation of reducing sugar in the blood results (W. R. Todd, J. Myers, and E. S. West, *J. Biol. Chem.* **127,** *275 (1939)*). V. P. Seeburg, E. B. McQuarrie, and C. C. Secor (*Proc. Soc. Exp. Biol. Med.* **89,** *303 (1955)*) have performed further studies on sorbitol administered intravenously in rabbits and report that an early fructosemia occurs which subsides rather rapidly after cessation of infusion. Blood glucose rises more slowly and continues to rise during the subsidence of the fructosemia.

Seeburg *et al.* administered the sorbitol at a rate of 1.0 g. per kilogram of body weight per hour and found the average urinary excretion of "carbohydrate" to be 17.7 per cent of the amount administered. About three fourths of the excreted material was unchanged sorbitol and one-fourth was fructose. At much slower rates of infusion (0.33 g. per kilogram per hour) the excretion of sorbitol was reduced, but not in proportion to the reduction in rate. The authors attribute this continued sorbitol loss in the presence of low blood sorbitol levels to lack of reabsorption of sorbitol by the renal tubules, as reported by W. W. Smith, N. Finkelstein, and H. W. Smith (*J. Biol. Chem.* **135,** *231 (1940)*).

The fact that sorbitol is not reabsorbed by the tubules and is not utilized by extrahepatic tissues suggests that this substance may have value as an osmotic diuretic. A. Leimdorfer (*Arch. internat. de pharmacodyn. et de thérap.* **100,** *161 (1954)*) studied this property of sorbitol in dogs and found that sorbitol injected intravenously provokes a marked increase in sodium, chloride and water output by the kidney. Leimdorfer postulated that sorbitol, by its osmotic effect, draws water from the intracellular compartment into the extracellular compartment, the blood volume increases and the protein concentration of the plasma is decreased. Renal plasma flow increases as does glomerular filtration rate, and diuresis ensues. According to Leimdorfer there are no signs of kidney damage after repeated injection of sorbitol. This is in contrast to the kidney damage seen following intravenous injection of sucrose.

Leimdorfer found that the combined administration of sorbitol and mercurial diuretics results in a strikingly increased excretion of water. When the two diuretics

were given together the urinary output was approximately doubled and the excretion of sodium and chloride was three times as great as that after sorbitol alone. Potassium losses were minimal.

The role of sorbitol as a dietary constituent has been discussed briefly in a previous review (*Nutrition Reviews* **12,** *178 (1954)*). Since it helps certain foods and pharmaceutical products retain moisture and improves their durability, sorbitol has been widely used as a "conditioning agent." Because it improves texture and because it is compatible with sweet foods (it is 60 per cent as sweet as cane sugar), sorbitol has been used in dietetic food products to increase palatability.

Of greater interest to physicians and nutritionists has been the potential usefulness of sorbitol as a constituent of the diet of diabetic patients. Since sorbitol is slowly absorbed from the digestive tract and is apparently converted to glucose only indirectly, the rate at which insulin is required to metabolize the carbohydrate derived from sorbitol should be considerably slower than the rate at which insulin is needed when common sugars are ingested. Although this possibility has been discussed at the theoretic level on many occasions, until recently very few clinical studies have been performed to determine the exact relation between sorbitol and insulin requirement.

In an attempt to throw further light on this problem, C. R. Shuman, R. L. Kemp, R. Coyne, and M. G. Wohl (*Am. J. Clin. Nutrition* **4,** *61 (1956)*) have made clinical observations on the effect of sorbitol feeding upon the metabolic status of 38 hospitalized diabetic patients. Sorbitol was administered in the form of an "ice cream" which contained 4 per cent fat, 4 per cent protein, 7 per cent carbohydrate from milk sources, and 18 per cent sorbitol. The ice cream was usually given in 200 g. quantities after lunch (36 g. sorbitol). In 29 of the patients the effect of sorbitol supplementation on blood sugar measured four times daily and glucose content of fractional urine samples was determined over a three- to five-day period.

In 6 patients whose diabetes was controlled by diet alone no alteration of blood glucose values occurred during the time that sorbitol ice cream was given as an extra feeding. Of the remaining 20 patients on insulin 6 required slightly higher insulin doses while receiving the sorbitol preparation. In general the insulin doses were so similar during the control and experimental periods that sorbitol was regarded as having virtually no effect.

In 9 other patients the effect of sorbitol administration on twenty-four-hour urine glucose excretion rates was determined. It is not clear how long these patients were studied in this manner. The mean glycosuria on the control diet alone was 7.4 g.; during sorbitol supplementation it was 6.5 g. In one patient the feeding of sorbitol ice cream had to be discontinued because of diarrhea. The other patients tolerated the preparation well.

In appraising the possible usefulness of sorbitol as an insulin-sparing carbohydrate supplement in the diabetic diet several important considerations must be taken into account. Because of its laxative effect only a limited amount of sorbitol can be consumed per day by the average patient. F. W. Ellis and J. C. Krantz (*J. Biol. Chem.* **141,** *147 (1941)*) determined the laxative threshold dose of sorbitol in 12 normal individuals, defining it as the minimum amount which produced very soft or watery feces. This result was obtained in the majority of subjects after oral administration of 20 to 30 g. of sorbitol syrup (83 per cent aqueous solution), and about 50 g. of crystalline sorbitol. Apparently, most subjects can tolerate well 40 g. (1.5 ounces) of sorbitol if it is spread throughout the day's intake of food.

The laxative effect of sorbitol is presumably due to the relatively slow rate at which it is absorbed from the small bowel (A. N. Wick, M. C. Almen, and L. Joseph, *J. Am. Pharm. Assn.* **40,** *542 (1951)*). In this regard it resembles oral fructose which also is rather slowly absorbed, inducing diarrhea by holding water in the intestinal tract. However, the slow rate of sorbitol absorption also may contribute to its value for diabetic patients since the rate at which it can be converted to glucose will necessarily be limited.

Evidence is accumulating that sorbitol is converted to fructose in the course of its metabolism. If this is the case, sorbitol, like fructose, is metabolizable in the liver of the diabetic patient without any need for insulin intervention. Accordingly, there are theoretic reasons for believing that sorbitol can be added to the diabetic diet in limited amounts without increasing the insulin requirement significantly. The preliminary findings of Shuman *et al.* appear to provide further support for such a possibility. Nevertheless, there is still need for more precise metabolic data concerning the nutritional value of sorbitol, both in normal subjects and diabetic patients. The studies of Shuman and coworkers were not performed under "metabolic-balance" conditions, and were too brief to be wholly convincing. Furthermore, it remains to be shown that the insulin-sparing effect of sorbitol in diabetic patients is sustained over a long period of time.

COPPER—A GROWTH PROMOTANT?

A tremendous amount of work has been done showing the growth-promoting effects resulting from the addition of small amounts of antibiotics, arsonic acids, and certain sulfonamides to commercial animal rations (*Nutrition Reviews* **11,** *208 (1953)*; **12,** *13, 108, 166, 336 (1954)*; **13,** *89, 169 (1955)*; **14,** *206 (1956)*). There have also been a number of reports suggesting that surface active agents have a similar action (C. M. Ely, *Science* **114,** *523 (1951)*; R. W. Luecke, J. A. Hoefer, and F. Thorp, *Mich. Agr. Exp. Station Quart. Bull.* **34,** *331 (1952)*). The wide variety of compounds which stimulate the growth of animals under farm conditions suggests that the mechanism whereby this effect is produced is probably a nonspecific one. The latter suggestion is supported by the observation of R. S. Barber, R. Braude, and K. G. Mitchell (*Chem. Ind., p. 601 (1955)*) that a mineral mixture which added small amounts of copper sulfate to a practical diet increased the growth rate of pigs.

An extensive evaluation of the stimulatory effect of the above mineral mixture on the growth of pigs was carried out in eight agricultural centers in England (R. J. Bowler *et al., Brit. J. Nutrition* **9,** *358 (1955)*). The pigs were fed the stock ration normally used at each of the stations. One group of animals received this ration, while littermate controls received the same ration to which was added 2.5 per cent of the mineral mixture. This mixture consisted principally of calcium carbonate, sodium chloride, and copper sulfate at such a level that it contributed 250 parts per million (p.p.m.) of copper to the diet. Small amounts of ferrous sulfate and traces of manganese sulfate, cobalt sulfate and potassium iodide were also present. Weanling pigs were started on the ration and maintained until they weighed approximately 180 pounds.

For the different experiment stations, the effectiveness of the mineral supplement in increasing the growth rate varied, but for a total of 182 animals studied the mean daily weight gain was 1.40 pounds for the controls and 1.48 for the mineral-supplemented pigs. This difference for the entire group was significant. There were slight but not significant improvements in the conversion of feed to body weight as a result of the mineral supplementation. There were no differences

in the carcass grades that could be attributed to the mineral supplementation.

In another report R. S. Barber, R. Braude, and K. G. Mitchell (*Brit. J. Nutrition* **9**, *378* (*1955*)) compared the growth-stimulating effect of aureomycin and the mineral mixture for pigs. They fed the control group a natural grain diet containing 10 per cent of fish meal. Other groups received the same ration supplemented with the mineral mixture previously used, an aureomycin concentrate, the mineral mixture plus the aureomycin concentrate, and copper sulfate in amounts equivalent to that in the mineral mixture. There were 6 weanling pigs in each group. These animals were kept on the experiment for fifteen weeks. At the end of that time, the group on the basal ration had gained 146 pounds, while those on the mineral mixture-supplemented ration and those on the copper sulfate gained 156 pounds. The aureomycin-supplemented pigs gained 163 pounds; those receiving both the mineral mix and the aureomycin concentrate gained 158 pounds.

Although the authors state that all supplements produced a significant increase in weight, their data indicate that only aureomycin produced a growth increase which was significant at the 0.1 per cent level. For the other groups receiving only mineral supplements, the weight increases were significant at the 5 per cent level. The latter is nothing more than suggestive, especially for groups as small as these. The results do, however, become important when it is realized that the earlier report from this group (*Chem. Ind., loc. cit.*) and the cooperative study (*Brit. J. Nutrition* **9**, *358* (*1955*)) all showed similar results for the mineral mixture.

The reports of the British workers bring to mind the work of L. E. Carpenter. He found that the addition of 4 ounces of copper sulfate per ton of a practical ration increased the weight of pigs from 153 pounds for those on the basal ration to 166 pounds (*Hormel Institute, Annual Report 1947–48, p. 21*). The copper also increased the litter size from 9.6 to 10.6. The addition of the copper sulfate to a practical ration had no influence on the growth of lambs. In the following year, Carpenter repeated his earlier studies on the growth-stimulating effect of copper sulfate for pigs (*Ibid. 1948–49, p. 23*). The latter work was done on a farm where the pigs had access to an alfalfa-sweet clover pasture at all times. The 110 pigs receiving the copper sulfate in the protein supplement gained 13 per cent more weight than those on the basal ration.

The amount of copper added to the ration by the British workers was approximately ten times that used by Carpenter. There is a possibility that the British group might have found the growth response with levels of copper as low as those used by Carpenter. The preceding assumption is based on Carpenter's observation that when the copper sulfate level was raised to that used by the British workers the resulting growth stimulation was the same as that secured at the lower level (*Capper's Farmer, Vol. 59, p. 24, Dec. 1948*).

That the action of copper in stimulating the growth of the pigs is not due to a mineral deficiency in the basal ration is shown by the presence of 11 micrograms of copper per gram of basal ration used by Carpenter (*Capper's Farmer, loc. cit.*). Such a level should provide two to three times the amount of copper proposed as being essential for growing swine (*National Academy of Sciences—National Research Council Publication No. 295, 5 (1953)*).

These findings indicate that inorganic as well as organic compounds may improve the rate at which animals raised under "farm conditions" gain weight. In view of the well known antimicrobial properties of copper, it is tempting to suggest that this may be the basis for its action in stimulating the growth of pigs. Carpenter entertained such a possibility when he made a number of bacteriologic examinations of the feces and intestinal contents of his swine (*Hormel Institute*

Annual Report 1947–48, p. 21). Since it is impossible to secure an exact profile of the microbial flora in the gastrointestinal tract and since the changes observed are difficult to interpret in terms of their potential influence on growth, bacteriologic studies are not likely to provide unequivocal answers as to how the antimicrobial agents increase the growth rate of animals. Nevertheless, most of the present evidence suggests that these substances improve growth as a result of their action on the intestinal tract.

VITAMIN K AND SULFAQUINOXALINE POISONING IN CHICKENS

The administration of sulfonamides to laboratory animals has been found to lead to nutritional deficiencies. The deficiencies that have been recognized include those of biotin, pantothenic acid, vitamin E, folacin, inositol and vitamin K. Disturbances in intestinal bacterial flora have been suggested as an explanation for this effect of the sulfonamides.

A condition characterized by hemorrhages in the body tissues and necrotic lesions in the spleen has in recent years occurred relatively frequently in poultry flocks, and it has been thought that the use of sulfonamides for the control of coccidiosis is etiologically implicated. The nature of the lesions in chicks receiving sulfonamides suggests a similarity to the multiple hemorrhages and increased blood clotting time characteristic of vitamin K deficiency in chicks (H. Dam, *Biochem. J.* **29,** *1273 (1935)*). This view is further supported by the observation that a hypoprothrombinemia in chicks induced by sulfamezathine could be corrected by the administration of synthetic vitamin K (F. D. Asplin and E. Boyland, *Brit. J. Pharmacol. Chemotherap.* **2,** *79 (1947)*).

Workers in Great Britain have recently investigated the relationship between blood clotting time and blood sulfaquinoxaline concentration in chicks receiving sulfaquinoxaline in drinking water at a level used to control coccidiosis (0.06 per cent). The effect of synthetic vitamin K (2-methyl-1,4-naphthohydroquinone) was also studied (L. P. Joyner and S. F. M. Davies, *J. Comp. Path.* **66,** *39 (1956)*). The effect of sulfaquinoxaline at different ages (1 to 67 days old) was tested in chicks from a common stock by administering the drug in drinking water for seven successive days. Death rate, edema, muscle hemorrhages and spleen lesions served as measures of the severity of reaction to the drug. Although blood clotting time was prolonged in birds of all ages as a result of the drug, the incidence of lesions and mortality was highest in chicks 4 to 8 weeks of age.

To explore the possibility that the drug followed a different pattern of absorption and excretion in younger birds, the study was repeated and the concentration of the drug in the blood was determined on the seventh day after beginning of administration. Although birds of all ages studied had about the same sulfaquinoxaline concentration in blood (range 7.9 to 9.9 mg. per cent), the birds in the 3 to 6 week age range were clearly exhibiting more severe symptoms.

Blood clotting times and sulfaquinoxaline concentration in blood were determined simultaneously in 12 6 week old chicks over a seven-day period. During this time the drug was given in the drinking water for a seventy-two-hour period. There was a close correlation between the concentration of the drug in the blood and the blood clotting time. Withdrawal of the drug was followed promptly by a reduction in the clotting time. This result is suggestive of a direct toxic effect on the blood clotting mechanism by the drug rather than an indirect inhibition of intestinal synthesis of vitamin K.

In further studies, three groups of 12 chicks were given the drug in drinking water for seven days. One group also received six

daily intravenous injections of 4 mg. of vitamin K_1, and a second group also received daily subcutaneous injections of 10 mg. of synthetic vitamin K analog. Although both vitamin preparations were effective in reducing blood clotting time, no striking reduction in incidence of spleen lesions was noted.

The authors conclude it is unlikely that sulfaquinoxaline toxicity in chickens is due solely to a shortage of vitamin K either because of inhibition of intestinal synthesis of the vitamin or because of a deficiency of the vitamin in the diet. They point out that under field conditions two hemorrhagic conditions may exist, one caused by vitamin K deficiency and the other by sulfaquinoxaline toxicity. The mechanism by which sulfaquinoxaline inhibits blood clotting, however, remains unexplained.

EFFECTS OF ANTIBIOTICS IN THE RUMINANT

A number of reports have indicated that antibiotics can be fed continuously with safety to beef cattle during the growing and fattening period, and that often such a supplement will prove profitable by increasing weight gain. When increased weight gains are observed, there is generally also an increase in the efficiency of feed utilization. This improvement in efficiency of feed utilization has never been adequately explained.

In digestibility studies with steers it was noted that aureomycin decreased fiber digestion (*Nutrition Reviews* **11,** *9* (*1953*)). Aureomycin was seen to suppress protein and crude fiber digestion to a greater extent than penicillin, although the two supplements had about equal effects in decreasing nitrogen retention (L. H. Horn, R. R. Snapp, and L. S. Gall, *J. Animal Sci.* **14,** *243* (*1955*)). In *in vitro* studies, E. M. Kesler (*Ibid.* **13,** *10* (*1954*)) found that rumen microorganisms from terramycin-fed steers digested less cellulose (24.2 per cent) than those from animals fed no antibiotic (77.4 per cent).

Aureomycin, bacitracin, terramycin and penicillin were found to depress the *in vitro* utilization of nonprotein nitrogen by the microflora of steers on grass-roughage feed (*Nutrition Reviews* **12,** *287* (*1954*)). Further digestibility studies with dairy calves have indicated little or no influence of antibiotics on the digestibility of nitrogen, ash, carbohydrates and ether extract of the ration (W. R. Murley, N. L. Jacobson, and R. S. Allen, *J. Dairy Sci.* **35,** *846* (*1952*); J. W. Hibbs, H. R. Conrad, and W. D. Pounden, *Ibid.* **37,** *724* (*1954*)).

Recently workers at the Kansas Agricultural Experiment Station have reexamined the apparent digestibility of certain calf rations as influenced by low levels of dietary aureomycin (E. E. Bartley, D. B. Parrish, and K. L. Wheatcroft, *J. Dairy Sci.* **39,** *319* (*1956*)). Six pairs of dairy calves were studied, one member of each pair receiving aureomycin hydrochloride at the rate of 25 mg. daily per 100 pounds of body weight. Digestion trials were conducted at 9 and 13 weeks of age.

When the calves received milk only, aureomycin slightly improved the digestibility of protein, ether extract, nitrogen-free extract and ash, but the differences were so small that they could not serve to explain the superior growth and feed efficiency that resulted from use of the antibiotic. When rations of milk and oats or milk and alfalfa hay were fed, there were no marked effects of aureomycin on the digestibility of nutrients, except for crude fiber. The antibiotic reduced the digestibility of this component in 13 week old calves fed milk and oats, and in 9 week old calves fed milk and alfalfa. At 13 weeks of age, aureomycin had no effect on crude fiber digestion when milk and alfalfa hay were fed. Since improved growth and improved feed conversion were apparent on all antibiotic-containing rations, the observed depression of crude fiber diges-

tion by the antibiotic is difficult to explain. Does this mean that the rumen fermentation, involving conversion of cellulosic materials to soluble nutrients, is a wasteful process with loss of net-energy value for growth purposes?

Further evidence that the *in vitro* rumen fermentation process is influenced by antibiotics is reported from Iowa State College (J. R. Lodge, J. T. Miles, and N. L. Jacobson, *J. Dairy Sci.* **39,** *303 (1956)*). Twenty lactating cows and 4 young calves (4 months old), half of which had received aureomycin from an early age, served as source material. Rumen fluid samples taken by stomach tube were centrifuged and microorganisms in the supernatant liquor served as inoculums in a mineral-cellulose medium. Cellulose digestion was measured by analysis of the medium in triplicate after a twenty-four-hour incubation. In samples from cows that had not received aureomycin, the average *in vitro* cellulose digestion was about 12 per cent higher than it was in samples from cows that had received antibiotic. Samples from calves receiving no supplement digested cellulose about 20 per cent better than samples from supplemented calves. One week after removal of antibiotic from the calf ration, however, the *in vitro* cellulose digestion in samples from supplemented calves exceeded those from non-supplemented animals by about 10 per cent.

Addition of aureomycin to the fermentation tubes revealed a marked difference between samples from aureomycin-fed and control cows. For example, 0.8 micrograms per milliliter of aureomycin added to fermentation samples from aureomycin-fed cows resulted in a depression of cellulose digestion from a level of 74.2 per cent to 72.0 per cent. Similar treatment of fermentation mediums from control cows reduced cellulose digestion from 86.5 per cent to 24.4 per cent. This observation indicated that either the organisms responsible for cellulose digestion in aureomycin-fed cows had adapted themselves or that new aureomycin-resistant populations had been established in such animals.

The persistence of aureomycin in the rumen was also investigated. Based on the amount of aureomycin fed, concentrations in the range of 2 to 3 micrograms per milliliter of rumen liquor were anticipated. Microbiologic assay for the antibiotic revealed that only about 5 per cent of this amount was present sixteen hours after feeding, and that only 10 per cent was present four hours after feeding.

No major differences between aureomycin-fed and control animals were detected with respect to rumen populations of total aerobes, total anaerobes, clostridia, lactobacilli, proteolytic organisms, coliforms, fungi, cellulose digesters, glucose fermenters, thermophiles and streptococci.

The relative sensitivity to aureomycin of selected groups of rumen organisms from aureomycin-fed and control animals was tested by plating on appropriate mediums with and without the addition of the antibiotic. Organisms from control animals appeared to be more sensitive to the antibiotic under these conditions than organisms taken from aureomycin-fed cows.

Although these results establish quite conclusively that aureomycin suppresses cellulose digestion when measured *in vitro* with rumen liquor as inoculum, the practical significance of this observation remains unanswered. N. L. Jacobson, J. T. Miles, and L. D. McGilliard (*J. Dairy Sci.* **37,** *657 (1954)*) and L. L. Rusoff and M. O. Haq (*Ibid.* **37,** *677 (1954)*) had previously shown that growth and efficiency of feed utilization were generally improved by the use of antibiotic, while milk production in dairy cows did not appear to be affected.

These results again emphasize that in terms of animal performance it is difficult to interpret data obtained with rumen microorganisms transferred from their natural rumen environment to the laboratory test tube.

NUTRITION STUDIES WITH THERMOPHILIC BACTERIA

As the nutrition of thermophilic bacilli becomes known in more detail, thermophiles (bacteria which grow at high temperatures) are emerging as possible tools for uncovering many metabolic pathways hitherto inaccessible to analyses by the microbial tools which have proved so successful with other systems, for example, B-vitamins, nucleic acid components and amino acids. A recent paper by H. Baker, S. H. Hutner, and H. Sobotka (*Ann. N. Y. Acad. Sci.* **62,** *349 (1955)*) describes the development of chemically defined culture mediums for representatives of several species including strains of *Bacillus stearothermophilus*. The basic incentive was to find means of assay for nutrients presenting special difficulties when supplied exogenously, such as high-molecular weight or fat-soluble substances.

For preliminary determination of nutritional requirements, the following basal medium was used: versen-ol (0.02 g.), potassium glycerophosphate (0.05 g.), magnesium sulfate·$7H_2O$ (0.04 g.), aconitic acid (0.5 g.), sodium acetate·$3H_2O$ (0.04 g.), glycerol (2.0 g.), DL-asparagine (0.25 g.), calcium (2.0 mg. as the chloride), molybdenum (2.0 mg. as the ammonium salt), vanadium (0.01 mg. as the sulfate), thiamine hydrochloride (0.2 mg.), niacin (0.2 mg.), biotin (4.0 micrograms), metals "ST" (1.5 ml. of the following mixture: ethylene diamine tetraacetic acid (EDTA), 2.5 mg. per milliliter; zinc, 4.0 mg. per milliliter; iron, 0.5 mg. per milliliter; manganese, 0.6 mg. per milliliter; calcium, 0.05 mg. per milliliter; cobalt, 0.02 mg. per milliliter; distilled water to 100 ml.; pH 6.9 to 7.1).

All of the 60 strains screened required biotin and most required, in addition, thiamine and niacin. These requirements are, in general, quite similar to *Bacilli* the growth optima of which are at lower temperatures. However, many strains of thermophiles had still additional requirements. One strain did not grow at all below 45°C., at 55°C. it required only biotin and at 75°C. histidine was required, and the addition of phenylalanine and glutamate further increased growth. Another strain grew well in the basal medium at 75°C. but required methionine, leucine and phenylalanine at 80°C. The general findings, in agreement with the results obtained at lower temperatures by L. L. Campbell and O. B. Williams (*J. Bact.* **65,** *146 (1953)*), were an increase in amino acid requirements with increasing temperature and an increase in the amount of substrate consumed in a given time. The latter finding implies a high degree of "permeability" in these organisms.

On the supposition that fat-soluble materials might be required at higher temperatures, bile was added to the basal screening mixture to serve both as a source of lipids and of emulsifying agents. As little as 10 mg. per cent of dehydrated ox bile was toxic at 55°C. However, in the presence of a "complete supplement," strain No. 1503 (National Canners Association) grew well at both 55°C. and 65°C. in the presence of 40 mg. per cent of ox bile. Analysis of the "complete supplement" revealed that DL-isoleucine (5.0 mg. per cent) overcame the toxicity of ox bile.

Although no enhanced growth from ox bile was noted, the above work cannot present a fair picture of the participation of lipids in thermophilly. These strains had been isolated on peptone and similar mediums which are virtually lipid-free. Also, the incubation temperatures were not pushed above 80°C., which would have increased the miscibility of lipids in the aqueous phase.

There exists other evidence that fatty acids have a special role in thermophilly. H. Sugiyama (*J. Bact.* **62,** *81 (1951)*) showed that when *Clostridium botulinum* was grown in 0.001 molar palmitate or oleate, the spores had a greatly increased thermostability. Although little is known of the fatty acid content of thermophiles as compared with

mesophiles (bacteria growing best at moderate temperatures), D. Dyer (Thesis, U. of Nebraska (1953)) found that the lipids of *B. stearothermophilus* were of the sphingomyelin rather than the lecithin type. Sphingomyelins are more stable to hydrolysis than are lecithins.

Even though thermophiles may eventually be found to utilize lipids in their metabolic systems, the relation of this to the metabolism of higher organisms must be critically evaluated. In most higher organisms lipids are readily metabolized at temperatures far below those optimal for thermophiles and it is quite possible that entirely different pathways prevail. It has been shown repeatedly that mammalian lipid metabolism varies markedly with localized temperature.

In an examination of the ability of *B. stearothermophilus* to utilize higher molecular weight substrates, several strains were encountered which responded to folacin. The requirement for folacin was also satisfied by para-aminobenzoic acid or by a combination of thymine, xanthine and cyanocobalamin. It was evident that a complete by-passing of para-aminobenzoic acid-folacin would entail the use of additional metabolites. Substantially the same growth effect was obtained when para-aminobenzoic acid was used in place of folacin. This equal effectiveness indicates that the para-aminobenzoic acid-pteroylglutamic acid requirement reflects a block in the synthesis of para-aminobenzoic acid. Bearing out this hypothesis, folinic acid, but not pteroic, was found to be active.

Strain No. 1503 (*loc. cit.*), which at 55°C. appeared to have absolute requirements for thiamine, niacin and isoleucine, grew densely when methionine was added. The methionine was replaceable by cyanocobalamin. This relationship between methionine and vitamin B_{12} is reported in detail by E. J. Ford and S. H. Hutner (*Vitamins and Hormones* **13,** *101 (1955)*). Again, the utilization of high molecular weight substances (such as vitamin B_{12}) reflects a high degree of permeability in these organisms.

Satisfactory explanations for thermophilly have not yet evolved. B. T. Stewart and H. O. Halvorson (*Arch. Biochem. Biophys.* **49,** *168 (1954)*) present evidence which supports the thermostable-enzyme theory. They found that racemase from spores of *B. terminalis* remained active after exposure to 80°C. for two hours. The corresponding enzyme from vegetative cells was almost completely inactivated in fifteen minutes. W. Militzer and L. Burns (*Ibid.* **52,** *66 (1954)*) found that while pyruvic oxidase from *B. stearothermophilus* was quickly inactivated at 60° to 65°C., malic dehydrogenase, cytochrome oxidase and aconitase were stable. However, the high concentration of growth factors required by thermophiles implies that their metabolism may be characterized by an accelerated wearing out of catalytic metabolites (dynamic theory of M. B. Allen, *J. Gen. Physiol.* **33,** *205 (1950)*). The enormous consumption of substrates by thermophiles is consistent with this idea. Quite possibly, both hypotheses may apply to some degree.

VITAMIN B_{12} AND SULFHYDRYL ENZYMES

The isolation of crystalline vitamin B_{12} led to the clarification of several problems posed by earlier investigations. For example, the vitamin was shown to be both the extrinsic factor and erythrocyte maturation factor described by Castle in studies of pernicious anemia. The injection of microgram quantities of pure vitamin B_{12} results in a satisfactory clinical response in pernicious anemia patients. Also, vitamin B_{12} appears to be the main component of the "animal protein factor" (*Nutrition Reviews* **6,** *219 (1948)*) shown to be required for normal growth and reproduction by several species of animals. The most recent advance in the vitamin B_{12} story was the elucidation

of the chemical structure of the pure compound (*Ibid.* **14,** *64* (*1956*)).

The most important unanswered question concerning vitamin B_{12} is its biochemical role in metabolism. One of the most promising leads appears to be the possible relationship of the vitamin to the metabolism of sulfhydryl compounds (*Nutrition Reviews* **11,** *304* (*1953*)). This group of compounds contains a functional sulfhydryl group which is capable of being reversibly oxidized to the disulfide form. One important example of such a compound in the body is glutathione. Perhaps more important, however, is the fact that many enzymes contain this sulfhydryl group which must be maintained in the reduced form for the enzyme to exhibit activity.

If vitamin B_{12} is required to maintain sulfhydryl groups in the reduced form, the vitamin should then affect the activity of a large variety of enzymes. This possibility has been investigated by J. W. Dubnoff and E. Bartron (*Arch. Biochem. Biophys.* **61,** *99* (*1956*)). These workers used a mutant of the microorganism *Escherichia coli* which required either vitamin B_{12} or methionine for growth. The organism was grown in the absence of vitamin B_{12} and the harvested cells were used for enzyme experiments. The effects of *in vitro* additions of vitamin B_{12}, reduced glutathione, and the combination of the two on a large number of enzymes were investigated. The results were quite consistent with the different enzymes, namely that the addition of either vitamin B_{12} or reduced glutathione activated the enzymes slightly while the combination of the two resulted in a marked increase in enzyme activity. This activating effect of vitamin B_{12} and reduced glutathione was observed on a variety of types of enzymes including dehydrogenases, splitting enzymes and transferring enzymes. These enzymes all were known to contain essential sulfhydryl groups.

This effect of vitamin B_{12} on such a variety of enzymes would seem to rule out its acting as a prosthetic group in the usual sense. Since the presence of reduced glutathione was necessary for the optimum effect of vitamin B_{12} in these experiments, and since the enzymes affected were known to be sulfhydryl enzymes, this vitamin B_{12} effect is most probably related to an influence of the vitamin on the metabolism of sulfhydryl compounds. Vitamin B_{12} may be related to a coenzyme concerned in maintaining sulfhydryl groups in the reduced form. Just how these *in vitro* effects of vitamin B_{12} may be related to its physiologic actions is not yet clear.

VITAMIN A AND CHOLESTEROL METABOLISM

Although the mechanism of action of vitamin A in scotopic vision (in dim light) is now fairly well understood, its more general growth-promoting and antixerophthalmic functions are still completely obscure. A hint that this vitamin may be connected with steroid metabolism was obtained by J. S. Lowe, R. A. Morton, and R. G. Harrison (*Nature* **172,** *716* (*1953*)) when an abnormal (probably steroid) constituent was found in the nonsaponifiable fraction of livers from vitamin A-deficient rats.

The possibility that steroid metabolism in general is deranged in vitamin A deficiency led these authors to investigate possible changes in plasma and liver cholesterol of deficient rats. Several groups of workers had previously attempted to follow changes in plasma cholesterol during development of the deficient state, but their results were inconclusive and conflicting. B. Green, J. S. Lowe, and R. A. Morton (*Biochem. J.* **61,** *447* (*1955*)) therefore designed an experiment, the results of which could be subjected to statistical analysis.

Forty rats, which had been maintained on a stock diet until 40 days old, were divided

into two groups on control and vitamin A-free diets. During the depletion period of up to nineteen weeks, 6 determinations of plasma cholesterol were made on each survivor. When deficiency symptoms became marked, terminal plasma and liver cholesterol and kidney vitamin A determinations were performed. No significant differences were found between plasma cholesterol levels of control and experimental animals at any stage or between terminal liver cholesterol levels of the two groups. It is evident that, if the abnormal metabolite is indeed derived from cholesterol, it represents a very minor pathway which exerts little influence on general cholesterol levels. Since the diets were virtually cholesterol-free, any appreciable alterations in endogenous cholesterol metabolism would have been apparent. Earlier results were readily explained by the extreme variance of serum cholesterol values of the experimental animals. Thus, with small numbers of animals, evidence could have been presented for an increased, decreased, or unchanged serum cholesterol in vitamin A deficiency.

The authors noted fatty livers in 3 of the experimental animals, 2 of which had the highest plasma and liver cholesterol values. It is possible that this deficiency is another factor in rendering the liver more susceptible to fatty infiltration. The negative results reported in this paper are not necessarily incompatible with the previous hypothesis that the production of the abnormal metabolite is a stage in the manifestation of vitamin A deficiency symptoms. It can be hoped that pursuit of this and similar lines of research will lead to elucidation of the mechanism of action of vitamin A.

CARBOHYDRATE METABOLISM OF THE LENS

The cataracts seen in diabetes mellitus and galactosemia implicate disorders of carbohydrate metabolism in the genesis of these changes in the lens. Only recently have there been any extensive studies of the carbohydrate metabolism of this tissue. J. Nordman, P. Mandel, and M. Achard (*Brit. J. Ophth.* **38,** *673 (1954)*) compared the effects of a variety of metabolic inhibitors on cataract formation using paired calf lenses. Lenses incubated in buffer solution alone exhibited no lenticular opacities over the period of study. However, all metabolic poisons that interfered with carbohydrate utilization produced cataracts. The inhibitors of glycolysis, such as fluoride, phlorhizin and iodoacetate, induced rapid formation of cataracts. Inhibitors acting in aerobic metabolism, such as malonate and fluoroacetate, produced cataracts more slowly. These results suggested that the integrity of the lens depends upon energy from both glycolysis and respiration involving the citric acid cycle.

J. E. Harris, J. D. Hauschildt, and L. T. Nordquist (*Am. J. Ophth.* **38,** *141 (1954)*) utilized another parameter for determining the effects of metabolic changes upon lens viability. When the lens is incubated at 0° C., potassium leaves the lens and sodium and water enter. Upon subsequent incubation at 37° C., these changes are reversed if the lens is viable.

These investigators found that glucose in the incubating medium at 37° C. was necessary for the reversal of the electrolyte changes. However, a high concentration of glucose in the medium (500 mg. per cent) reduced the degree of cation recovery. This effect was shown not to be due to osmotic forces. Furthermore, the deleterious effect of the high glucose medium could be nullified by addition of adenosine triphosphate (ATP).

This same group of investigators studied the transfer of glucose into the intact lens (J. E. Harris, J. D. Hauschildt, and L. T. Nordquist, *Am. J. Ophth.* **39,** *No. 2, Part II, 161 (1955)*). The glucose in the lens was never higher than it was in the medium.

The absence of oxygen or the presence of cyanide inhibited the transfer of glucose into the lens. The presence or absence of the lens capsule did not seem to affect the final glucose concentrations. This type of study is complicated by the utilization of glucose and the suggestion that the fluid within the lens is not homogeneously distributed. Thus, more weight should be given to the effects of inhibition of oxygen utilization than to final glucose concentrations. It is therefore suggested that the transport of glucose into the lens is an active metabolic process.

Z. Dische and G. Ehrlich (*Am. J. Ophth.* **39,** *No. 2, Part II, 99 (1955)*) investigated the utilization of various intermediates of glycolysis by the lens capsule. Glucose, glucose-6-phosphate and fructose-1,6-diphosphate were metabolized at equal rates both aerobically and anaerobically. Dische and Ehrlich showed that adequate levels of ATP were necessary for utilization of the glucose. They also indicated that the hexose monophosphate shunt could not be quantitatively important because of lack of utilization of ribose and adenosine.

Similar studies by H. Green, C. A. Bocher, and I. H. Leopold (*Am. J. Ophth.* **39,** *No. 2, Part II, 106 (1955)*) were performed in the cell-free supernatant of homogenized lens. Glycolysis was demonstrated to be linked to the presence of inorganic phosphorus and this was a limiting factor. When glucose-6-phosphate or fructose-6-phosphate was substituted for glucose, the rate of formation of lactic acid was doubled. Thus, the limiting reaction was the hexokinase reaction. The addition of ATP did not increase lactic acid formation, but small amounts of muscle hexokinase added to the system yielded large increases in reaction rates.

Finer details of carbohydrate metabolism were probed by J. H. Kinoshita (*Arch. Ophth.* **54,** *360 (1955)*), using C^{14}-labeled intermediates. He first noted that the lens was very nearly unable to utilize pyruvate, as indicated by the presence of only small amounts of labeled carbon dioxide after incubation of 2-C^{14} pyruvate with whole bovine lenses. The lenses did catalyze the conversion of pyruvate to lactate without any utilization of either component. It was concluded that the citric acid cycle was relatively unimportant.

Because of this, further studies relating to the hexose monophosphate shunt were undertaken. It was first noted that the lens and the lens capsule contained glucose-6-phosphate dehydrogenase and 6-phosphogluconic acid dehydrogenase. As both of these enzymes are important in the shunt, their presence suggested that the shunt might be operative.

Studies using glucose labeled at either C_1 or C_6 were performed to quantitate the amounts of glucose metabolized via the shunt. It is apparent that if the shunt mechanism is used, then the C_1 of glucose will appear as carbon dioxide earlier than the C_6. If, however, all the glucose were metabolized through the Embden-Meyerhof route, then C_1 and C_6 should appear as carbon dioxide simultaneously. The lenses were incubated with either C_1- or C_6-labeled glucose. The experimental utilization of these labeled sugars indicated the hexose monophosphate shunt to be of considerable quantitative importance. With further measurements of the formation of labeled lactate from 1-C^{14} glucose and 6-C^{14} glucose, it could be calculated that for every 4 moles of glucose utilized by glycolysis, 1 mole of glucose was metabolized through the direct pathway. Thus, with respect to the total metabolism of glucose, the shunt is relatively important and it has been shown to be more active than the citric acid cycle in the oxidative metabolism of glucose.

These studies represent some of the initial steps leading to an understanding of lens metabolism. The lens, chiefly because of its avascularity, is a good experimental object for biochemical investigation, and its reactions in diseases of carbohydrate metabolism open another field of interest common to the biochemist and physician.

NUTRITURE AND TOBACCO SMOKE LESIONS IN MICE

S. J. Kreshover (*J. Am. Dent. Assn.* **45,** *528 (1952)*) has studied the influence of tobacco smoke on certain epithelial tissues of mice fed normal and vitamin-deficient diets. Tobacco smoke from a lighted cigarette was applied to the lower lip or the ear by a specially designed suction device in which a small glass tube was held lightly against the tissue and the flow of smoke over the tissue was controlled by means of a pinch valve. The smoke was allowed to pass at a standard rate over the experimental area for a two-second period. This was followed by a three-second stagnant period during which the smoke was kept in contact with the area. A waiting period of twenty-three seconds was then allowed before the smoking procedure was repeated on the same area. Ten applications were made to the specific area of the lower lip and five to one ear of each mouse on alternate days. Altogether 78 mice were used for this study with smoke applications extending over a seventy-six-day period. Twenty mice were maintained on a normal diet, 8 on a vitamin A-deficient diet and 22 on a vitamin B-complex-deficient diet. In addition, 18 gonadectomized mice were fed a normal diet and 10 a vitamin B-deficient diet.

When examined grossly, the smoked ears of the animals on the normal diet showed little evidence of pathologic change beyond a slight degree of thickening and redness. This minimal change was all that was observed even after 38 smoke applications covering the whole experimental period. Little information was obtained from the vitamin A-deficient group, since half of these animals died prior to the twentieth day of the experiment. None of the ears of the vitamin A-deficient group showed any change beyond that observed in the group maintained on the adequate diet. However, the findings in both the vitamin A-deficient mice and the normal mice were in striking contrast to the results in those that were maintained on a vitamin B-complex-deficient diet. After only twenty days unmistakable evidence of tissue change was observed. The ears were markedly thickened, scarred and in many places excoriated. In the group that was gonadectomized and maintained on an adequate diet, there was almost a complete absence of ear changes regardless of the number of smoke applications. Again, in the gonadectomized mice maintained on the vitamin B-complex-deficient diet, the changes noted were of minimal nature comparable to those in the intact mice fed the normal diet. Gross examination of the lips showed no significant changes in any of the experimental animals regardless of the group.

These studies have been expanded to include experiments in which mice were fed diets deficient in either thiamine, riboflavin, pyridoxine, pantothenic acid, niacin, or biotin (S. J. Kreshover, *J. Dent. Res.* **34,** *798 (1955)*). A total of 201 Swiss strain mice between the ages of 10 to 12 weeks were used in this study. The same apparatus was used for applying whole tobacco smoke to the right ear of each mouse during the eighty-day experimental period. In all cases the smoke application was standardized so that on alternate days the mice received a like number of repeated applications of equal volume, exposure time and interval periods. A smoke flow of 17.5 cc. per second was selected arbitrarily and applied against the ear for a five-second exposure period, which was repeated five times on each day that an application was made. The mice were observed over a five-month period.

A series of control animals was tested to determine the possible influence of pressure and vacuum during the test procedure by placing the applicator against comparable areas of the ear when an unlighted cigarette was in the apparatus. Another group was used to test the possible influence of elevated temperature on the tissues of the ear. The tests for the influence of suction and pressure alone did not produce any pathologic changes. It was noted in the examination of

temperature effects that there was a temperature rise of only 1.8° C. in the smoke-application tests, which was believed to be insufficient to cause any changes.

As in the previous experiment the animals maintained on the normal diet showed few changes. The maximal ear changes in this group consisted of a slight degree of thickening and an alteration in color and texture which imparted a yellow-brown, parchment-like appearance. Histologically the epidermis on the smoked side of the ear showed hyperplasia as well as some keratinization, a round cell infiltration with edema, increased vascularity and fibrosis. These lesions did not progress after the smoke applications were stopped during the additional three-month study period. As in the previous study, mice that were deficient in several members of the vitamin B-complex exhibited a much greater tissue susceptibility to whole tobacco smoke than did normal animals. The changes consisted principally of ulceration, induration, excoriation and thickening which became visible as early as the fortieth day of smoke application, that is, twenty days in which smoke treatments were given. Thickening was most pronounced in the area bordering the ulcers, contributing to a rolled and raised margin in the lesion. Over these areas there was a thick deposit of amorphous material comprised of tobacco smoke and serous exudate. There was no evidence of an intrinsic capacity of the tissue for progressive breakdown or growth after the smoke applications were stopped on the eightieth day. Healing occurred in some animals to a minimal degree; however, definite acceleration of repair was induced when deficient animals were returned to a normal diet.

When the individual deficiencies were studied, it was observed that riboflavin, pyridoxine and pantothenic acid deficiencies resulted in the most definite initiation of lesions which were comparable in degree to those in the group fed the vitamin B-complex-deficient diet. However, the thiamine, biotin and niacin deficiencies did not result in any predisposing influence on the tissue response to whole tobacco smoke.

In this particular series of experiments, less emphasis was placed on the relationship of castration to the tissue changes, and only 6 mice in the riboflavin-deficient group were gonadectomized. In this case there was a moderate thickening of the ear due principally to increased epithelial keratinization and epithelial hyperplasia. These changes were much more like those seen in the mice fed the normal diet than in those fed the riboflavin-deficient diet.

In the discussion the author mentioned that a large group of hamsters was currently under study. These animals were divided into various experimental categories somewhat comparable to the mouse experiments and received whole tobacco smoke applications for prolonged periods with the sites of exposure being the oral mucosa and the ear. At the end of 80 successive daily applications, the tissues of vitamin B-complex-deficient animals showed practically no gross evidence of pathologic change in either of the treated areas. Kreshover points out that this striking species difference from what had been observed in mice may well be of sufficient importance to be taken into account in the evaluation of the data on the mouse.

The fact that vitamin B-complex deficiency, and in particular riboflavin, pyridoxine and pantothenic acid deficiencies, increased the tendency for whole cigarette smoke applications to cause deleterious changes in the ears of mice is worthy of considerable additional study. The retarding influence of gonadectomy also needs to be studied at length in order to understand the mechanism by which the tissues in the gonadectomized but vitamin-deficient animals had as good resistance to the smoke applications as did normal animals.

One of the main problems in these two papers that is worthy of additional consid-

eration is the difference in susceptibility of the oral tissues of the mouse from those of the ear. Presumably the lack of lip lesions is due to the accessibility for cleaning the lip after the smoke applications with the resultant dilution of the toxic materials by the saliva and the reduced period of exposure on the lip. On the other hand, this difference between tissues may not be entirely explainable on the basis of differential cleaning but possibly some difference exists in the degree of resistance of these two tissues to the smoke applications, predetermined on the basis of such things as rate of blood flow and other similar physiologic phenomena.

The role of nutrition in the production and prevention of these lesions is not clear but is possibly related to the maintenance of systemic reactions that are vital for the growth and repair of epithelial structures. Whether the lesions observed in the mouse have any relevance to human problems is of less importance than the fact that a test mechanism is available for the determination of the lesions that are produced and how they may be resisted. The question frequently arises whether data obtained in one species can be translated into information that is relevant to another strain within the same species, to another experimental species and ultimately to human beings. This is one price that has to be paid in the use of experimental animals but one that over the years has yielded abundant dividends.

RIBOFLAVIN BIOSYNTHESIS

Riboflavin is known to be synthesized by many species of bacteria. This biosynthesis is a commercial source of the vitamin. The influence of medium composition on the synthesis of riboflavin by bacteria has been studied (*Nutrition Reviews* **12,** *48 (1954)*), but little information is available concerning the metabolic origin of the various carbon atoms of the riboflavin molecule. Such information could aid in the design of an optimum medium for riboflavin production by bacteria and of course would also be of great value as fundamental information.

The isotopic tracer technic, so successful in elucidating the structure of the purine ring, has been applied to this problem. G. W. E. Plaut (*J. Biol. Chem.* **208,** *513 (1954)*; **211,** *111 (1954)*) and Plaut and P. L. Broberg (*Ibid.* **219,** *131 (1956)*) have studied riboflavin biosynthesis by *Ashbya gossypii*. This microorganism was grown on a medium of natural materials to which were added various simple compounds labeled with radioactive carbon (C^{14}). The riboflavin synthesized by the bacteria while in contact with the labeled material was then isolated. The molecule was degraded and the radioactivity of the various carbon atoms was determined. The radioactive compounds tested included methylene- and carboxyl-labeled glycine, methylene- and carboxyl-labeled acetate, formate, bicarbonate, totally labeled glucose, and glucose labeled in the 1, 2, or 6 carbons.

In the first experiments the carbons of ring C of the riboflavin molecule were studied. It was found that formate was an excellent precursor of carbon 2, none of the other compounds tested contributing significantly to this carbon. Carbon 4 was found to be derived from bicarbonate and to a lesser extent from carboxyl-labeled acetate. It seems likely that the acetate was oxidized to carbon dioxide and this metabolic carbon dioxide was then incorporated into the carbon 4 of riboflavin. Carbons 4A and 9A, which are shared by rings B and C, were liberated together in the degradation procedure. They were found to be derived about equally from carboxyl- and methylene-labeled glycine.

It is probable that the methylene carbon of glycine serves as a precursor for one of these carbons and the carboxyl carbon for the other.

Attention was next directed to the carbons of ring A of the riboflavin molecule. The earlier experiments had shown that all the radioactivity of the riboflavin molecule produced by the bacteria in the presence of radioactive glycine or formate could be accounted for by ring C, hence these compounds were not precursors of any of the carbons of ring A or of the ribityl side chain. It was found that either glucose 1-C^{14} or glucose 6-C^{14} was a good precursor of carbons 5 and 8 and of the two methyl carbons attached to ring A of the riboflavin molecule. Methylene-labeled acetate was also a good precursor of these carbons, suggesting that glucose was incorporated via a two-carbon fragment. Carbons 6 and 7 and 8A and 10A were found to be derived from the carboxyl carbon of acetate. The latter two carbons are shared by rings A and B of riboflavin.

These experiments elucidated the precursors of the carbons of the rings of riboflavin, and the final paper was concerned with the metabolic origin of the side chain. It was found that glucose carbons were good precursors of the ribityl carbons. The pattern of incorporation of glucose labeled in various positions was not consistent with a single known pathway of carbohydrate metabolism. Most probably the organism employed in these experiments metabolizes glucose via a variety of pathways.

These experiments elucidate the metabolic origin of the carbon atoms of the riboflavin molecule. The various carbons are derived from glycine, formate, bicarbonate, acetate and glucose. The next logical extension of this work would be a study of the intermediate compounds in the synthetic pathway.

THE DISTRIBUTION OF EXCESSIVE WATER

In this era of extensive investigation into the distributions of a variety of substances among the determinable body compartments, the distribution of water itself warrants more study. It was proposed by J. L. Gamble, G. S. Roos, and F. F. Tisdall (*J. Biol. Chem.* **57,** *633 (1923)*) that the cells of the body acted as perfect osmometers and that water was distributed between cellular and extracellular compartments in accordance with the distribution of osmotically active particles. If this theory is correct, then the addition of a known amount of water to the body should cause predictable alterations in vascular solute concentrations.

This hypothesis was tested by J. R. Elkinton, A. W. Winkler, and T. S. Danowski (*Yale J. Biol. Med.* **17,** *383 (1944)*), who infused large loads of 5 per cent glucose in water into 3 dogs. In 2 of these dogs, the total extracellular fluid base was appreciably lower than the value predicted. This predicted value was based on a distribution of the excess water throughout the total body water. Subsequently, Elkinton, Winkler, and Danowski (*J. Clin. Invest.* **26,** *1002 (1947)*) utilized changes in the chloride space to follow the partition of infused water between intracellular and extracellular fluid. Because more water remained in the chloride compartment than was predicted, they postulated "inactivation" of intracellular osmotically active constituents.

A. Leaf, J. Y. Chatillon, O. Wrong, and E. P. Tuttle (*J. Clin. Invest.* **33,** *1261 (1954)*) studied in dogs the mechanisms of osmotic adjustment of the body cells to a large water load. This was done by determining the distribution of a large water load and simultaneously measuring the total body water by deuterium oxide dilution. From the change in solute concentration and knowledge of the total body water,

it is possible to establish whether the administered water was distributed in a manner proportional to the distribution of total body water. In order to achieve appreciable water retention, the dogs were nephrectomized or had their ureters ligated. Some animals received large amounts of pitressin in lieu of surgery. The changes in total solute were corrected for the concentration of glucose, making the assumption that the glucose remained in the extracellular fluid.

When the distribution of the water load was calculated on the basis of the change in concentration of either serum sodium or total solute, the mean volume of distribution of water approximated the values for total body water. The conclusion justified by this study was that the osmotic adjustment of the body cells *in vivo* involves movement of water into cells so that the load is distributed throughout the total body water.

As noted by Leaf and co-workers, these results are compatible with another hypothesis; namely, that when the water load is administered, solutes are extruded from the cells. However, unless these solutes were sodium alone, the volume of distribution of water based on sodium concentration would be lower than that based on total solute concentration. If sodium were extruded from bone where it is unaccompanied by chloride, there would be a greater dilution of serum chloride. However, the changes in serum chloride were, in fact, quite variable, probably depending on the volume of gastric juice. In two instances the drop in serum chloride was identical with the fall in serum sodium. Thus, it seemed unlikely that extrusion of sodium from body or cellular compartments contributed to the osmotic adjustments to the water load.

Similar experiments were performed by V. Wynn (*Clin. Sci.* **14,** *669 (1955)*). Water retention was produced in a healthy human subject by a combination of prior electrolyte depletion and oral water loading while pitressin tannate was given. Total body water was estimated by antipyrine dilution. In the subject who was studied, the plasma sodium decreased 15 milliequivalents (mEq) to 123 mEq per liter in a period of nine hours. Symptoms of water intoxication were manifested. The distribution of the water load was calculated to be throughout a space essentially equal to the measured total body water.

The same type of study was carried out in dogs with ligated ureters. The values for the extracellular fluid base after excess water administration were close to predicted values, the mean difference between observed and predicted final total base concentration being 0.6 milliosmols per liter. The plasma chloride levels, however, always fell more than predicted. This, in part, could reflect loss of chloride in gastric secretions, although other chloride shifts may play a part. Wynn concluded that the classic "Gamble" theory of water distribution fitted the experimental data and that there was no satisfactory evidence to substantiate the suggestion of "inactivation" of intracellular, osmotically active constituents.

Related to the problem of water distribution are the clinical aspects of water intoxication. V. Wynn and C. G. Rob (*Lancet* **I,** *587 (1954)*) discussed in some detail the hypotonic syndromes. They pointed out that a low serum sodium occurs in three separate situations, sodium depletion, water intoxication, and in the symptomless hypotonicity most frequently noted in the chronically ill patient. If it is accepted that excess water distributes itself throughout intracellular and extracellular compartments in accordance with the volume of these two compartments, then it can be calculated that the addition of only 3.6 liters of water would cause a drop of 10 per cent in the serum sodium level of the average human adult. If, in addition, 140 mEq of base have been lost, only 2.6 liters of water will be required to effect this same change in serum sodium.

Thus, small losses of base and moderate water retention may suffice to cause moderately severe water intoxication.

Water intoxication was most frequently noted after surgery or following rectal administration of water. The symptoms are nonspecific and similar to those seen with sodium depletion—lethargy, anorexia, muscle weakness, apathy and disorientation. The physical signs, however, are different from those of sodium depletion. Weight is not lost, tissue turgor is normal, the skin is warm and the blood pressure is normal.

Wynn and Rob quoted the histories of several cases and discussed their treatment. Usually, water restriction alone will suffice to restore normal electrolyte levels. However, in the acute or dangerous situation, hypertonic saline may be given in amounts calculated to raise the serum sodium to safer levels. The amount of excess water necessary to produce symptoms of water intoxication varies among patients. There is a suggestion that acute lowering of plasma solutes is not so well tolerated as a more gradual onset of water intoxication. It is probably dangerous to exceed a 10 per cent lowering of the plasma osmotic pressure.

FUNCTION OF VITAMIN D

Classically, vitamin D function has been considered mainly in its relationship to calcification and enhancement of the gastrointestinal absorption of bone salts. Early reports of the beneficial effects from the addition of citrate to rachitogenic diets led to the observation that vitamin D administration to rachitic infants markedly increased urinary citrate excretion and elevated serum citrate levels (*Nutrition Reviews* **10,** *233 (1952)*). Similar increases in tissue citric acid and augmented excretion of this acid have been demonstrated in rachitic rats receiving vitamin D (S. Freeman and T. S. Chang, *Am. J. Physiol.* **160,** *341 (1950)*; S. A. Bellin and H. Steenbock, *J. Biol. Chem.* **194,** *311 (1952)*; Steenbock and Bellin, *Ibid.* **205,** *985 (1953)*).

Relationships between bone salt deposition and soft tissue growth in young rats as they are influenced by dietary calcium, phosphorus and vitamin D were the objects of a study reported by H. Steenbock and D. C. Herting (*J. Nutrition* **57,** *449 (1955)*; *see also Nutrition Reviews* **14,** *191 (1956)*). Young rats (70 to 80 g. body weight) were fed diets containing 0.018 per cent of calcium and nine different levels of phosphorus, ranging from 0.016 to 1.168 per cent. All mineral levels were compared in three-week feeding tests with and without the addition of vitamin D_2. Without vitamin D addition, growth decreased as the phosphorus in the diet was increased. The addition of vitamin D not only prevented this inhibition but stimulated growth considerably. For example, rats fed the vitamin D-low diet containing 0.296 per cent of phosphorus gained 13 g. in three weeks, contrasted with a gain of 96 g. when vitamin D was added.

In prolonged studies it was shown that rats receiving vitamin D continued to grow for ten weeks, whereas death occurred in half the animals on the vitamin D-low diet in about nine weeks. Tetany, diarrhea, dry scaly paws and tails and bloody nares were seen in the animals that succumbed on the vitamin D-low diets.

The feeding of high-phosphorus, low-calcium diets induced an elevated blood phosphate level (11.2 to 14.9 mg. of phosphorus per 100 ml. of serum) that was corrected when vitamin D was included in the diet.

Despite the considerable growth stimulation from vitamin D, the ration was so low in calcium that bone calcification could

not take place. For example, an initial femur organic weight (difference between dry weight and weight of bone ash) of 0.054 g. increased to about twice this value when the rats had been on vitamin D-containing diets for three weeks whereas the bone ash (initially 64 mg. per femur) did not increase appreciably even in animals that gained as much as 30 to 40 g. per week in body weight. The authors logically raise the question as to the validity of body weight gain as a reliable criterion of nutritional health in view of the above cited results.

Another interesting observation was that the addition of vitamin D to the low calcium diet rather consistently reduced bone calcification, as evidenced by reduced femur ash content. Disregarding the lowest phosphorus diet (0.016 per cent) where bone ash values were very low (25 per cent ash versus 54 per cent for normal bone), the 47 animals on varying phosphorus levels averaged 45.8 per cent ash in femur when no vitamin D was added to the diet. Those with added vitamin D averaged 41.4 per cent ash.

Despite the very low calcium level in the diet, serum calcium levels were normal in the animals receiving vitamin D, whereas hypocalcemia was common when vitamin D was omitted.

The effect of varying the amount of calcium, in the presence of 0.315 per cent of dietary phosphorus, was also studied in the presence and absence of dietary vitamin D. Calcium levels studied ranged between 0.21 and 2.63 per cent of the diet. While increasing dietary calcium improved growth considerably when no vitamin D was fed, this effect was shadowed by the real growth stimulation from vitamin D at all dietary calcium levels up through 0.83 per cent. On high dietary calcium levels vitamin D actually suppressed growth. Bone ash was normal at all calcium levels when the diet contained vitamin D. In the absence of the vitamin, values 10 to 20 per cent below normal were consistently observed.

Since dietary calcium alone elicited only about one-half the growth response that was obtained from vitamin D alone on low calcium diets, Steenbock and Herting consider this strong evidence that this vitamin performs a specific metabolic function other than its influence on the absorption and deposition of bone salts. A stimulatory effect on the Krebs tricarboxylic acid cycle with a resultant increase in soft tissue growth was postulated as a possible vitamin D function and is supported by the evidence already cited of increased urinary citric acid following vitamin D administration (*Nutrition Reviews, loc. cit.*).

D. C. Herting and H. Steenbock (*J. Nutrition* **57,** *469 (1955)*) also investigated the effect of vitamin D on gastric secretion. Following a ten- to twenty-four-hour fast, gastric secretions collected through a pyloric ligation were found to be more acid in animals on rachitogenic diets than in animals on normal diets. The increase in gastric acidity was correlated with an increase in serum phosphorus.

Although the results of Steenbock and Herting suggest strongly that vitamin D plays an important role in the growth of soft tissue, it is entirely possible that the action of the vitamin in this role could still be mediated through its influence on calcium or phosphorus utilization. It would be of interest to determine the total body calcium retention in the presence and absence of vitamin D under dietary conditions that permit marked growth stimulation from this vitamin.

NOTES

Dietary Fat and the Nephrotic Syndrome in Rats

The nephrotic syndrome in man is characterized by a renal lesion, albuminuria, hypoalbuminemia, hyperlipemia and cholesterolemia, and massive edema and ascites when it is "full blown." A disease closely resembling the nephrotic syndrome in man can be produced in rats by the injection of a rabbit anti-rat kidney serum (W. Heymann and H. Z. Lund, *Pediatrics* **7,** *691 (1951)*). In these animals hyperlipemia and hypercholesterolemia are regularly observed. M. Friedman, R. H. Rosenman, and S. O. Byers (*J. Clin. Invest.* **33,** *1103 (1954)*) have studied the effect of high and low fat and cholesterol diets in these animals compared to normal rats.

Thirty rats were studied of which 10 were controls fed the stock ration. Twenty were given the anti-rat kidney serum and of these 7 were fed a fat- and sterol-free diet before and after the injection. Eight were fed the stock laboratory ration the same as the controls and 5 the stock ration with a supplement of cholesterol and cholic acid. Determination of total cholesterol and total lipids in the serum showed that the treated rats fed the fat- and sterol-free diet developed hyperlipemia and hypercholesterolemia to the same extent as those ingesting the stock ration. This was further increased by cholesterol and cholic acid feeding.

The authors believe that the hypercholesterolemia was due to inability of the liver to remove plasma cholesterol and not to an increase in rate of production of cholesterol by the liver. The data necessary to support the hypothesis that cholesterol is not manufactured by the liver in increased amounts in this circumstance are not yet published.

It would seem from these observations that the characteristic turbid serum due to high fats and cholesterol in the nephrotic rat is not due to excessive cholesterol feeding nor yet to overproduction of these substances by the liver, but presumably to failure of their removal by the liver. Just how far these studies may be carried over to nephrosis in man is, of course, unknown, but the close resemblance of the condition in rats to the human disease makes such a carry-over seem reasonable.

Vitamin Retention in Frozen Foods

Studies of vitamin destruction during food preparation have been concerned largely with cooking and storing procedures. Methods of freezing and thawing have received less attention despite their increasing importance. A. M. Pearson and co-workers (*Food Research* **16,** *85 (1951)*; *Nutrition Reviews* **9,** *255 (1951)*) found that from 8 per cent (folacin) to 33 per cent (pantothenic acid) of the vitamins present in beef cuts before freezing were lost during storage and thawing of the frozen meat. The major loss was laid to the drip, which occurred during the latter part of thawing. F. A. Lee and co-workers (*J. Am. Dietet. Assn.* **30,** *351 (1954)*; *Nutrition Reviews* **12,** *288 (1954)*) reported no significant change in the vitamin content of pork chops during six months storage at 0°F. or below.

L. H. Kotschevar (*J. Am. Dietet. Assn.* **31,** *589 (1955)*) has measured losses of thiamine, riboflavin and niacin in calves' liver, sliced or ground, during all combinations of the following procedures: (1) freezing (0 to 5°F.) for forty-eight hours; (2) storage in polyethylene wraps at the above temperature for sixty days; (3) thawing (four hours at room temperature or twenty hours at 40°F.); (4) cooking (five to six minutes in margarine with or without thawing). Adequate replication of samples was carried out with triplicate analyses for each sample.

For the sliced liver, some of the more important statistically significant results were as follows: Freezing with forty-eight-hour storage caused no loss of vitamins.

Cooking caused about 15 per cent loss in thiamine and riboflavin. Storing for sixty days caused a 43 per cent loss in thiamine and 41 per cent loss in riboflavin. Thawing caused a loss in thiamine. For the ground liver, similar results were obtained, with the exception that less thiamine was lost during thawing.

A consideration of the problem of drip revealed that with the liver slices drip amounted to 8 to 15 per cent of the weight of the meat and had approximately the same vitamin content per gram as the remaining meat. This is a significant loss for the sliced liver but is negligible for muscle meats of various types since the per cent of drip was much smaller in these cases. As meat thaws, the temperature rises rapidly to the point at which the ice crystals melt and remains at this temperature for some time before a further rise occurs. Drip becomes appreciable at the start of this second temperature rise and can be largely prevented by cooking at this point.

Recent Nutrition Books

Nutrition Sourcebook. Oliver E. Byrd. Stanford University Press, Stanford, California. London: Geoffrey Cumberlege, Oxford University Press. Pp. 370. Price: $7.50.

Infant Nutrition in the Subtropics and Tropics. D. B. Jelliffe. World Health Organization, Monograph Series No. 29, Geneva, 1955. Published by Columbia University Press, New York 27, N. Y. Pp. 237; 24 illustrations + 2 plates. Price: $5.00.

Feeding Your Baby and Child. Benjamin Spock and Miriam E. Lowenberg. Duell, Sloan and Pearce, New York, Boston; Little, Brown & Company, Canada. Pp. 213. Price: $3.75.

Dry Milk Products. A Symposium sponsored by the Quartermaster Food and Container Institute. Advisory Board on Quartermaster Research and Development, Committee on Foods, National Academy of Sciences—National Research Council, Washington, D. C., July 1955. Pp. 194.

The National Food Situation. NFS-74. (1956 Outlook Issue). Can be obtained from The Agricultural Marketing Service, U. S. Department of Agriculture, Washington 25, D. C. Pp. 31.

How to Reduce Surely and Safely. Herbert Pollack, with Arthur D. Morse. McGraw-Hill Book Company, Inc., New York, Toronto, London. Pp. 152. Price: $2.95.

BOARD OF TRUSTEES

JOSEPH F. ABBOTT, *Chairman of the Board, American Sugar Refining Co.*

PAUL S. ARMSTRONG, *General Manager, Sunkist Growers*

A. C. BENJAMIN, *President, Junket Brand Foods, Chr. Hansen's Laboratory, Inc.*

*F. G. BOUDREAU, *President, Milbank Memorial Fund*

CHARLES S. BRIDGES, *President, Libby, McNeill & Libby*

G. BUITONI, *President, Buitoni Foods Corp.*

*CASON J. CALLAWAY, *Hamilton, Georgia*

*O. C. CARMICHAEL, *President, University of Alabama; Chairman of Board of Trustees, The Nutrition Foundation, Inc.*

THOMAS P. CARNEY, *Vice President, Research, Development & Control, Eli Lilly and Company*

GEORGE H. COPPERS, *President, National Biscuit Company*

E. B. COSGROVE, *Chairman of the Board, Green Giant Company*

JOHN N. CURLETT, *President, McCormick & Co., Inc.*

WESLEY M. DIXON, *President, Container Corporation of America*

ROGER DRACKETT, *President, Drackett Company*

*LEE A. DUBRIDGE, *President, California Institute of Technology*

*CHARLES WESLEY DUNN, *New York, New York*

J. S. ELLITHORP, JR., *President, Beech-Nut Packing Company*

THOMAS C. FOGARTY, *President, Continental Can Co., Inc.*

EDGAR J. FORIO, *Vice President, The Coca-Cola Company*

*CLARENCE FRANCIS, *New York, New York*

DANIEL F. GERBER, *President, Gerber Products Co.*

*RUFUS C. HARRIS, *President, Tulane University*

HENRY J. HEINZ, II, *President, H. J. Heinz Co.*

*REV. THEODORE M. HESBURGH, C.S.C., *President, Notre Dame University*

JOHN HOLMES, *Chairman of the Board, Swift & Company; President, The Nutrition Foundation, Inc.*

J. M. KAPLAN, *President, Welch Grape Juice Company, Inc.*

RIDGWAY KENNEDY, JR., *Chairman, Abbotts Dairies, Inc.*

*JAMES R. KILLIAN, JR., *President, Massachusetts Institute of Technology*

*CHARLES GLEN KING, *Executive Director, The Nutrition Foundation, Inc.*

*GRAYSON L. KIRK, *President, Columbia University*

JAMES E. KNOX, *Chairman, Chas. B. Knox Gelatine Co., Inc.*

R. NEWTON LAUGHLIN, *President, Continental Baking Co.*

J. PRESTON LEVIS, *Chairman, Owens-Illinois*

R. G. LUCKS, *President, California Packing Corp.*

W. F. MCLEAN, *President, Canada Packers, Limited*

HANFORD MAIN, *President, Sunshine Biscuits, Inc.*

ARMAND MAY, *Chairman, American Lecithin Co., Inc.*

OSCAR MAYER, *Chairman of the Board, Oscar Mayer & Co.*

JOHN T. MENZIES, *President, The Crosse & Blackwell Co.*

GEORGE W. MERCK, *Chairman, Merck and Co., Inc.*

JOEL S. MITCHELL, *President, Standard Brands, Inc.*

CHARLES G. MORTIMER, *President, General Foods Corporation*

MILTON C. MUMFORD, *Executive Vice President, Lever Brothers Co.*

W. B. MURPHY, *President, Campbell Soup Co.*

H. EARLE MUZZY, *Vice Chairman, The Quaker Oats Company*

JOHN C. NAYLOR, *Executive Vice President, Pet Milk Company*

*THOMAS PARRAN, *Dean, Graduate School of Public Health, University of Pittsburgh*

RUSSELL G. PARTRIDGE, *Representing United Fruit Company*

H. L. SIPPLE, *Secretary*

PHILIP W. PILLSBURY, *Chairman, Pillsbury Mills, Inc.*

J. G. PLEASANTS, *Vice-President in charge of Research and Development, The Procter & Gamble Company*

L. RATZESBERGER, JR., *President, The Illinois Canning Co.*

ERNEST W. REID, *Chairman, Corn Products Refining Co.*

B. E. RICHMOND, *Executive Vice President, Richmond-Chase Company*

R. A. ROBERTSON, *President, and Managing Director, George Weston, Limited*

ROBERT B. SCHNERING, *President, Curtiss Candy Company,*

MILTON L. SELBY, *President, Safeway Stores, Inc.*

ELBERT M. SHELTON, *Secretary, The Baker Laboratories, Inc.*

WALTER SILBERSACK, *President, American Home Foods, Inc.*

*FRANKLYN BLISS SNYDER, *Evanston, Illinois*

FREDERICK W. SPECHT, *President, Armour & Company*

ALFRED N. STEELE, *Chairman, Pepsi-Cola Company*

W. C. STOLK, *President, American Can Company*

VERNON STOUFFER, *President, The Stouffer Corporation*

*LEWIS L. STRAUSS, *New York, New York*

G. CULLEN THOMAS, *Vice President, General Mills, Inc.*

L. A. VAN BOMEL, *Chairman, National Dairy Products Corp.*

W. H. VANDERPLOEG, *President, The Kellogg Company*

R. D. L. WIRTH, *President, Red Star Yeast and Products Co.*

H. J. WOLFLISBERG, *President, The Nestle Company, Inc.*

C. I. WOOD, *President, Thomas J. Lipton, Inc.*

HARRY W. ZINSMASTER, *President, Zinsmaster Baking Co.*

---

* Representative of the public.

THE NUTRITION FOUNDATION

The Nutrition Foundation was organized by food and related manufacturers in December, 1941, as a sincere expression of their interest in scientific progress and human health. The basic purposes of the Foundation are: (1) the development of a comprehensive program of fundamental research, providing basic information in the science of nutrition; and (2) the support of educational measures that will assist in making the science of nutrition effective in the lives of present and future generations.

The publication of NUTRITION REVIEWS has been undertaken to enable professionally trained people to keep abreast of current progress and to have available an unbiased, authoritative review of the current research literature in the science of nutrition.

The Editorial Staff has been carefully selected so that the publication will merit, in reasonable degree, the confidence of the medical profession and others who work directly with the public in the field of nutrition. The service provided by the publication is distinct from that of an abstract journal, a review for laymen, or an extensive scientific review. Since the reviews as originally prepared by a member of the Editorial Staff are frequently modified by special referees and may be further modified by the Editorial Office in preparing final copy for the press, the Editorial Committee feels that it is preferable not to affix the names of Editorial Staff members to individual reviews. There is thus a high degree of group responsibility on the part of the Editor and Associate Editors.

Signed articles do not necessarily represent the opinion of the Editorial Staff.

A leaflet based on articles appearing currently in NUTRITION REVIEWS and entitled "Current Research in the Science of Nutrition" is distributed by The Nutrition Foundation each month. The vocabulary of the leaflet is adapted for lay reading and the subject matter is restricted to clearly established findings of practical interest. Subscriptions should be sent to The Nutrition Foundation, Inc., 99 Park Ave., New York City. Rate $1.00 per year in the United States and Canada; $1.25 in other countries.